F. C. Bishopp

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SOME PROBLEMS IN MEDICAL AND VETERINARY ENTOMOLOGY

F. C. BISHOPP*

In Charge, Division of Insects Affecting Man and Animals, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture

The field of medical and veterinary entomology presents innumerable problems of intense scientific interest and of great importance to the welfare of man.

A review of the accomplishments in this field and an enumeration of their direct and indirect values to the human species would provide material for several addresses of greater length than should be presented even though one may have the advantage of an unrestricted period on this program. Too much credit cannot be given to the pioneers in this field; those illustrious scientists who by their untiring efforts, deductive reasoning, and unfaltering courage proved the relationship between arthropods and disease, and pointed the way by which these vicious chains might be broken and man freed of many of his most devastating scourges and heaviest economic burdens. Literally hundreds of scientists have made substantial contributions to the solution of these problems. It is difficult without the perspective which comes only with the lapse of time, and a most searching analysis, to evaluate the results of research or to rank the contributors according to the influence of their lives and works on the welfare of mankind. Certain men, such as Manson, Ross, Bruce, Grassi, Laveran, Reed, Smith, Kilborne, and Howard stand out clearly as having made monumental contributions to our knowledge of medical and veterinary entomology. Scores of others whose works may not have been so conspicuous may deserve ranking as close seconds to these.

These men had markedly different background, training, and method of approach, but each exhibited persistence, sound judgment, clear reasoning, and above all an insatiable desire to learn the truth. One is tempted, when one's mind is allowed to contemplate the accomplishments of these outstanding men of science, to enter into an historical discussion and to eulogize those who were foremost in laying the foundation for the science of parasitology.

*Address of the Retiring President, American Society of Parasitologists, December 29, 1938, Richmond.

This I shall leave to someone better versed in the history of science and for presentation at a more appropriate time, perhaps at a meeting celebrating the Silver Anniversary of our Society or of the founding of the JOURNAL OF PARASITOLOGY, of which we have every reason to be proud.

I would prefer, with your leave, to browse about in the broad field permitted by the title, and hope that perhaps some of the commonplace statements which I shall make regarding medical entomology may have useful application in other parasitological fields.

For convenience I shall treat the subject from three main viewpoints, viz., teaching, research, and application.

The success of efforts in any one of these fields is largely dependent upon cooperation. Many of the world's most important health problems have an important entomological phase; perhaps none of them are wholly entomological. Life processes and relations are so complicated that not one but a great many factors must be considered in making a successful approach to any one of them. The field to be covered is so broad that no individual or group of individuals specializing along one line can be expected to arrive at a complete and thoroughly satisfactory solution of any major problem in medical entomology, or, for that matter, in any other broad field involving living creatures. Cooperative attack on these problems is the answer, but we all know the difficulties that lie in this direction even though there are many examples of close and highly effective joint effort. Although there are still opportunities for the exceptional individual research worker, this is the day of massed and properly coordinated attack. Even the most pronounced individualist finds himself dependent upon many other previous and contemporary workers, and his production can attain a maximum of reliability and perfection only when his efforts are supported by others. In the broad field of teaching, and especially in the application of research results, cooperation becomes imperative.

It is true that the failure of scientists working in related fields to cooperate is frequently not chargeable to a disinclination to do so, but rather to the preoccupation of the individual in his specialty. This difficulty, especially in larger organizations, may be overcome in part at least by joint consideration and planning of projects on a long-time basis with coordinators of broad training and vision to see that effective cooperation is arranged so that each group will make its special contribution to the solution of the problem.

There is a need for men especially trained in veterinary and medical entomology. Unfortunately little effort appears to have been made in our colleges and universities to provide curricula calculated to produce men satisfactorily trained for the conduct of research in this field.

Courses in entomology have been designed mainly for the training of agricultural entomologists. Likewise most veterinary and medical courses give little consideration to the parasitological field, or to the many subjects urgently needed by the research man in medical entomology.

In extension work, also, this field has not received the attention it deserves. Many problems relating to annoying and disease-transmitting insects can be met only by arousing public interest to the point of concerted effort. In such cases the success of control procedure is dependent upon effective extension work. Many problems in medical entomology lend themselves well to publicity but in my opinion not one-fourth of the attention is given to publicizing research and control results in the field of medical entomology as they might profitably receive. This seems to me to be true of most lines of work in which members of our society are engaged.

I quite agree with Dr. Tyzzer's statement in his presidential address before this society a few years ago that "parasitology has not received its share of publicity" and that "the life histories of many well-known parasites and insect pests, such as fleas, mosquitoes, black flies, and midges, would be of great general interest were they properly presented, and there is no reason why the study of the distribution and the life histories of some of these forms should not be undertaken as hobbies." The collection and study of such insects of direct importance to the comfort and health of man would appear to be as intriguing a hobby as that of more showy forms of life such as beetles and butterflies. Interest in such studies implanted in children is likely to crop out in adult life in the form of hobbies or in a desire to assume leadership in community effort in combating undesirable and dangerous insect pests. Strange to say, however, a great many of our young people finish grade school, high school, and even college without being exposed to one brief course in any phase of parasitology. I believe there is need for a textbook on parasitology suitable for grade and high school students, and that this society might well take the initiative in getting included in the curriculum of every high school in this country an elemental course in a subject which touches, often in a vital way, the life of every individual.

Relatively few of the medical entomological problems are sufficiently understood to enable us to lay down sound recommendations for their practical solution. There are many others regarding which not even elemental knowledge is available.

Many do not regard taxonomic investigations as part of the work of the medical entomologist, while others consider insect classification and identification as his major work. Whole groups of arthropods such as ticks, lice, fleas, and mosquitoes are of great importance as disease

vectors, hosts of other organisms of medical importance, parasites, or annoyers of higher animals. The importance of a knowledge of the phylogenetic relationship of such groups is unquestioned as is also the importance of accurate identification of species and races. A lack of information of this sort may be responsible for long delays in the determination of insect-disease relationships and even abort efforts to cope with a disease or parasite problem. A failure to have insect vectors properly identified may render almost valueless otherwise excellent pieces of research work. Furthermore, research and especially control operations based on an erroneous identification of forms are likely to cause wastage of large amounts of money and reflect adversely on other sound projects.

The failure for many years to identify properly the American screwworm fly, it seems to me, is a good example of this. In 1933 Cushing and Patton found by a careful study of the genitalia of the flies that for many years two distinct species had been consistently recognized as one and the same, and that apparently one of these is an obligatory parasite of higher animals while the other is largely a scavenger. Subsequent investigation definitely confirmed this and made necessary a review of many lines of work upon which much effort had been previously expended, and, what is more important, control recommendations had to be modified.

Biological and control work on insects which affect man and animals would be materially furthered by a considerable enlargement of taxonomic research. It is important to be able to identify accurately the adult forms as well as the eggs, larvae, and often the pupae. Frequently attack in control operations is directed at the immature stages rather than the adult. Immature stages may be difficult to rear and such rearing may result in long and costly delays. On the other hand, in certain instances identification of immature stages of certain insects may be easier and more certain than that of the adults of those species. Obviously the phylogenetic relationships of insects can best be established by having available for study all the stages. Knowledge of the true relationships of insects, as well as other forms of life with which the parasitologist deals, is likely to have a number of applications to strictly practical problems.

Ordinarily we do not think of physiological investigations as being of great importance in connection with medical entomology. Certainly a knowledge of the physiological reactions of the host to its parasites is of great value in the development of methods of host protection. Likewise investigations of the physiology of the insects which attack higher animals or transmit diseases is of decided importance in determining the true relationship between host and parasite, and in developing control measures.

As a homely example, we are often asked why carbon monoxide is not recommended for the control of insects such as fleas in garages. The layman, in suggesting this method of attack, fails of course to realize the great difference between the physiology of respiration in insects and that in warm-blooded animals—that insect blood, devoid of haemoglobin as it is, does not have the oxygen exchange blocked by the carbon monoxide as in higher forms of life.

Investigations of the physiology of blowfly larvae and the nature of their excretions has led to the discovery by William Robinson of the value of allantoin and urea in the stimulation of healing in indolent lesions caused by such conditions as osteomyelitis, extensive burns, diabetes, and varicose veins.

As illustrative of the relation between the physiology of insect vectors and their disease-transmitting potentialities reference is made to the determination, by H. Mooser and M. R. Castaneda, that although the virus of Mexican typhus multiplies abundantly in several species of fleas, those insects are less effective than lice in transmitting the disease. One reason assigned by those investigators for this phenomenon is that the peritrophic membrane in the fleas apparently prevents the free discharge of the rickettsiae into the lumen of the gut and consequently the feces of the fleas is less infectious than that of the lice.

Although habits and incidence of insects are of paramount importance as factors influencing their part in the transmission of disease, the physiology and also the anatomy of such insects may have a distinct bearing and should receive more consideration than has been given heretofore.

Recent investigational work indicates that a study of the physiology of insects may suggest why certain insecticides are ineffective and point the way to the development of insecticides that are of low toxicity to warm-blooded animals but are more effective against resistant insects.

Biological studies of insects which attack man and animals have rightfully received much attention in the past, yet there are hundreds of species important as parasites or as disease bearers the life histories of which have not been worked out. A reasonably accurate knowledge of the essential features of the life histories of such forms is of basic importance. It is neither necessary nor desirable that biological studies of a species await the demonstration that the species is a serious parasite or the vector of a disease. Such studies often are essential to an establishment of causal relations with disease. Many of the most knotty problems in clearing up insect-disease relationships are associated with the development of technique for the successful rearing of the insects; witness the impetus given to malaria investigations by the development by M. F. Boyd of a special cage for rearing *Anopheles quadrimaculatus*. A

cage of sufficient height to provide for the mating swarm was the essential feature which had prevented previous investigators from successfully rearing this scourge of the human race.

In the case of species incriminated in disease causation, broad biological investigations are essential. Ecological aspects of many of the important insect problems have received too little attention, and apparently this criticism applies equally well to most studies of living things. Under pressure to secure practical results, we are inclined to think only of points of direct attack and to neglect studies which might indicate indirect though perhaps simple ways of coping with a destructive species. We must have knowledge of how a species fits into the normal biological complex and what changes from the normal have brought the species into economic prominence in order to suggest counter changes that may be made to suppress it. The normal physiological and biochemical processes and histological conditions in an organism must be understood if the effect of disease or parasitism is to be evaluated. In the interpretation of information along many of these lines statistical treatment has its place. This appears to be true particularly when many different factors are being considered, as in an ecological study.

The assembling of information on the distribution, seasonal incidence, and annual abundance of the many species of arthropods which affect higher animals is of great value in epidemiological studies of various diseases the etiology of which has not been fully determined.

I have generalized at some length on the needs of medical and veterinary entomology and on suggestions relative to procedure. There are so many important specific problems which demand investigation that it has not appeared desirable to attempt even an enumeration of them.

A good test of the soundness and adequacy of research results is to attempt to apply those results on a large scale. Furthermore such large-scale control efforts often yield valuable basic information. The close coordination of research and control activities is therefore necessary to the best interests of each line of work.

Problems in the control or eradication of insect pests or disease carriers, in common with all problems involving man, are heavily charged with the human equation. This element enters the picture so strongly that it is often dominant and frequently becomes the most difficult phase of the problem. Man is so constituted that he does not want to have his preconceived ideas or his usual modes of living and carrying on business interfered with, and he is likely to resist any effort which impinges upon his activities. The solution of the human phase of the problem is usually dependent upon education, and thus control efforts and all forms of extension work must proceed hand in hand.

Recommendations for the control of insects must be sound, logical, and, above all, practical. This means that they must be firmly founded

on facts and involve no half-baked theories. One of the major problems before us today is to get people who are confronted with an insidious menace to recognize it and take action, and on the other hand to get those who are seriously beset with an insect pest or disease to support research and wait patiently for the development of a practical solution.

Insects and related forms are so numerous and present such diversity in their life histories and habits that it is impossible to lay down general rules for control procedure. Usually each problem must be considered separately and lines of attack adopted which take full advantage of the vulnerable points in the biology of the insect, due consideration being given to the cost of control or eradication as weighed against the economic importance of the pest.

Among important general factors influencing the methods and vigor of attack on insects affecting man and animals are host relations, adaptability of the species to environmental conditions, degree of dependence on higher animals, continual or intermittent parasitism, distribution and dispersion tendencies, and importance of the species as a parasite or as a transmitter of diseases or parasites of higher animals.

One problem encountered in the control of any pest or disease is that of restriction of spread. The rapid transportation systems and widespread movements of people and commodities under modern conditions give abundant opportunities for the scattering of pests and diseases and challenge the ingenuity of those responsible for the protection of the health and welfare of man. A realization on the part of the traveler and shipper of these dangers is necessary to the successful operation of quarantines and the development of more effective methods of detecting and destroying insects at points of embarkation or in transit.

In this connection emphasis should be placed on the protective value of reducing the number of insects in the vicinity of commercial centers, and especially near points of call of transoceanic or intercontinental air lines. The reduction of the numbers of an insect pest which has strong migratory tendencies is often an aid in reducing the rapidity and extent of dissemination. The difficulty of controlling species given to migration is obvious. It should be borne in mind that many insects are to be found drifting in the upper air, and indications are that in this way deleterious species may traverse tremendous distances, also that wingless insects or weak fliers are the dominant forms in the upper air.

In prosecuting control campaigns against insect-borne diseases there are several possible lines of attack, such as destruction of the disease organism in the higher animal host, immunization of susceptible hosts, prevention of infection by isolating those hosts which are disease carriers, protection of the uninfected from the insect vectors, and destruction of the insect vectors. Usually no one of these lines of attack is adequate and a combination of several of them must be resorted to. This is

well illustrated by the malaria problem. Theoretically the anophelines concerned can be controlled, the disease organisms destroyed in the human host by the use of quinine and synthesized drugs, and spread of infection prevented by protection of diseased and susceptible people from mosquito attack. In practice each of these lines of attack fails and combinations of all of them have been none too successful in wiping out malaria.

Many arthropod vectors of disease are themselves important as parasites or annoyers; therefore, the control or eradication of such vectors has a practical advantage over other methods of coping with the disease.

The example set by the work of the Bureau of Animal Industry and cooperating states in solving the splenetic fever problem by eradicating the tick vector may well be followed in the case of other disease carriers and troublesome pests of man and animals. The eradication from large areas or from the entire country of such pests as lice of poultry and livestock, human lice, horse bots, cattle grubs, and certain mites and ticks appears not to be out of the question even if the main reliance is placed on control measures now available.

There are, to be sure, many obstacles to be overcome in large scale control operations—witness the difficulty encountered in cattle fever tick eradication in Florida owing to the large number of deer there and their ability to perpetuate the tropical form of the cattle tick in pastures containing no livestock or in which domestic animals are dipped regularly.

In applying control measures against parasites or disease-bearing forms, careful consideration should be given to the question as to whether the measures used are inimical to other interests of mankind.

As an illustration reference is made to the poison-residue problem which is related to the field of medical entomology. The attack of destructive insects on many food crops and the popular demand for practical freedom from insects or evidence of their work on fruit and vegetables have led to the free and persistent use of insecticides. Among these the arsenicals and fluorine compounds have been widely employed against such insects as the codling moth, the celery leaf-tier, the cabbage worms and the tomato fruit worm.

The finding of dangerous quantities of arsenic on farm products offered for sale, the occurrence of cases of acute illness resembling poisoning followed by seizures and condemnations of large quantities of cabbage, cauliflower, and celery by officials of the U. S. Department of Agriculture and by state health authorities, and the appearance of many published articles on the dangers of consuming foods bearing insecticides led to widespread alarm and a serious effort to remove or otherwise prevent poisonous residues and to find materials of high toxicity to insects and low toxicity to man. Even the use of Paris green in the control of malaria mosquitoes drew adverse comment.

The development of pyrethrum and tobacco dust mixture for the control of the celery leaf-tier, of derris or cube against the Mexican bean beetle, the turnip aphid and the raspberry fruit worm, and of various dust and spray mixtures to replace arsenicals in the control of other fruit and vegetable pests, and the development of more effective methods of removing residues are long steps in meeting this serious problem.

Just now much interest is being shown in wildlife preservation and restoration, and the whole-hearted way in which mosquito control has been pushed forward by ditching marshes has received the bitter condemnation of certain wildlife enthusiasts. Possibly neither the mosquito control workers nor those interested primarily in wildlife preservation have fully appreciated the others' viewpoint, but in any case it illustrates the point that before large-scale control measures are inaugurated careful consideration should be given not only to the effect of such control upon the pest in question, but also to its effect upon other forms of animal and plant life which may be useful to mankind.

In conclusion I should like to reemphasize the interdependence of research, educational work, and control operations in the field of medical and veterinary entomology and the important part cooperation plays in the success of each. This statement, I believe, applies with equal force to any phase of parasitology.

DESCRIPTIONS OF FIVE NEW SPECIES OF FLAGELLATE PROTOZOA OF THE GENUS *GIARDIA**

BERNARD V. TRAVIS**

A number of species of the genus *Giardia* have been described from vertebrate hosts. This paper includes descriptions of five new species of *Giardia* taken from four species of birds, American bittern, little blue heron, western meadowlark, and swamp sparrow, and from one mammal, the muskrat. The species recorded here were studied both while alive and in Schaudinn-iron-hematoxylin preparations. Type slides are kept in the author's collection, in the Department of Zoology, Iowa State College, Ames, Iowa, and in the U. S. National Museum, Washington, D. C.

The measurements were all made by means of the camera lucida from fixed and stained material. The figures of the trophozoites are ideal drawings taken from the dimensions as determined by measuring 10 specimens. The orientation and position of the various structures were verified with the camera lucida.

Giardia botauri n. sp.

(Figs. 1, 2; Table 1)

One specimen of the American bittern, *Botaurus lentiginosus* (Montagu) (type host), was collected at Ruthven, Iowa, during the summer of 1932, and another was taken in the fall of 1934 from the same locality. Both specimens harbored a species of *Giardia* in the small intestine which appears to be distinct from flagellates of this genus studied by other authors. The mean dimensions for 100 individuals are, 6.6×10.4 μ; the range in width, 5.5–8.0 μ, and in length, 8.0–13.5 μ. One cyst, 7.6×9.5 μ, with four nuclei was observed (Fig. 2).

This species is similar in size to the species of *Giardia* of the black-crowned night heron, *Nycticorax nycticorax hoactli* (Gmelin), which Hegner (1925) considers to be *G. ardeae* (see table 1). The species in the bittern is apparently slightly narrower in proportion to its length than is *G. ardeae,* the ratio being 1.6 as compared to 1.4 for *G. ardeae* from the night heron. The distance from the anterior end to the center of the nucleus is almost the same as in *G. ardeae,* the distance from the center of the nucleus to the posterior end of the lateral shields is somewhat less, and the distances from the ends of the lateral shields and across the lateral

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** Formerly with the Iowa Agriculture Experiment Station, now with the U. S. Bureau of Entomology and Plant Quarantine.

shields are greater. The body shape of these two species is quite similar, except that *G. botauri* is narrower across the center of the nuclei and broader across the lateral shields.

The sucker disc averages 6.0 μ for 10 specimens, with a ratio of 1.7 of the length of the body to the length of the sucker. The nuclei often reach the posterior edge of the sucker disc.

The parabasal bodies are not observable in all the specimens of *Giardia* from the bittern. When present, they appear as a pair of short, blunt rods, which lie dorsal to and at an angle with the axostyle. The rods, which are located between the nuclei, vary in position from a point on a line with the bottom of the nuclei to a point about on a line with the center of the nuclei. This species also differs from *G. ardeae* in that the nuclei (1.6 × 2.6 μ) retain a more anterior position.

Type slide: U. S. Nat. Mus. No. 9158.

Giardia floridae n. sp.

(Fig. 3; Table 1)

This species of *Giardia* was found to be present in the small intestine of two individuals of the little blue heron, *Florida caerulea caerulea* (L.) (type host), collected at Ruthven, Iowa, during the fall of 1934. The mean dimensions for 100 specimens are 5.7 × 9.1 μ; range in width 4.5–6.5 μ, and in length 7.5–10.5 μ. The measurements and morphology are unlike those of any of the previously described species of *Giardia* from avian hosts.

The width across the posterior ends of the lateral shields is much less than in *Giardia botauri*, being 2.8 μ for *G. floridae* as compared to 3.6 μ for *G. botauri*. The body shapes of the species from the night heron, little blue heron, and the bittern are quite similar.

Giardia floridae differs from other species of the genus taken from herons and bitterns in the morphology of the parabasal apparatus and in the dimensions of the body. A species reported by Hegner from the great blue heron does not seem to possess parabasal bodies. This apparatus appears to be present as one rather large ellipsoidal body in the case of *G. floridae*, whereas in the *Giardia* from the night heron and the bittern there are two blunt rods. In a few cases the parabasal body in the flagellates from the little blue heron appeared to be split slightly at one end, indicating the possibility that there are two closely appressed bodies instead of one.

The nuclei (1.5 × 2.4 μ) lie near the posterior edge of the sucker disc. The sucker disc is 5.3 μ in length or a ratio of 1.7 with the total length of the body. No cysts were observed.

Type slide: U. S. Nat. Mus. No. 9155.

Giardia sturnellae n. sp.

(Fig. 5; Table 1)

In the spring of 1931 a crippled western meadowlark, *Sturnella neglecta* (Aud.) (type host), was brought to the Zoology Department of Iowa State College, Ames, Iowa. This bird was found clinging to the radiator of a car that had come from Nebraska. Upon examination, giardias were found in the small intestine. No cysts were observed.

This is one of the smallest giardias reported from avian hosts. The mean dimensions for 100 individuals are 5.7×9.1 μ; range in width 4.5–7.0 μ, in length 7.0–11.5 μ. The general shape of the body and the dimensions compare more nearly with *G. muris* than any of the other previously described species. The distance from the center of the nuclei to the lateral shields and to the end of the body is greater than in *G. muris*.

The sucker disc averaged 4.5 μ in length for 10 specimens, resulting in a ratio of 2.0 with the total length of the body. This is a rather large ratio for giardias from birds. The nuclei (1.5×2.0 μ) lie near the posterior edge of the sucker disc.

The parabasal bodies are short, blunt rods that frequently lie parallel to the axostyles, but usually are at an angle with the latter structures.

Type slide: U. S. Nat. Mus. No. 9156.

Giardia melospizae n. sp.

(Fig. 4; Table 1)

One specimen of a swamp sparrow, *Melospiza georgiana* (Latham) (type host), collected during the summer of 1932 was heavily infected with giardias. This appears to be the first record of a flagellate of this genus occurring in sparrows, a group in which few intestinal flagellates have ever been reported. No cysts were observed. The mean dimensions for 100 individuals are 6.0×9.0 μ; range in width 4.0–7.5 μ, in length 6.5–12.5 μ.

This, along with *Giardia sturnellae*, is the smallest of the genus described from avian hosts, and shorter than any other giardias, except the form reported by Hegner (1923) from wild mice near Baltimore. In this paper Hegner reported the mean length of 8.8 μ and 9.0 μ for *Giardia muris* from two wild mice.

The ratio of width of the body to the body length is 1.5, a ratio which compares favorably with the giardias of man. The sucker disc averages 4.9 μ in length for 10 specimens or a ratio of 1.8 with the total length of the body. The nuclei (1.4×2.3 μ) lie near the posterior margin of the sucker disc.

The two parabasal bodies are short, blunt rods which typically lie at an angle with the axostyles.

Type slide: U. S. Nat. Mus. No. 9157.

Giardia ondatrae n. sp.

(Fig. 6; Table 1)

A wandering muskrat, *Ondatra zibethicus* (L.) (type host), was captured in the spring of 1933 on the Iowa State College campus, Ames, Iowa. The small intestine of this animal was found to contain a species of *Giardia* that is apparently unnamed. During the winter and spring of 1934–35, four muskrats from the lake on the college campus were examined, and three were infected with this same species of *Giardia*. Fifteen muskrats were collected from the lakes near Ruthven, Iowa, during the spring and summer of 1935; eleven were found to harbor this flagellate. Bishopp (1934) reported *Giardia* from a muskrat in Europe, but gave little of the morphology of the species. No cysts were seen on the author's slides. The mean measurements of 100 specimens are 6.4 × 11.7 μ; range in width 5.0–8.0 μ, in length 9.0–15.0 μ.

This species is quite similar to *Giardia microti* from field mice, *Microtus* spp. The total length and the width across the center of the nuclei compare favorably with the measurements of *G. microti*. The width across the lateral shields and the width across the center of the nuclei are slightly less, making the organism appear more slender than the giardias from mice. The ratio of length to width for *G. ondatrae* is 1.8 and for *G. microti* 1.5. Additional measurements between the various body structures vary somewhat between the two species (see table 1).

The sucker disc averaged 6.1 μ in length for 10 specimens, making a ratio of 1.9 to the total length of the body. The sucker disc on *G. microti* averages 5.3 μ in length with a ratio of 2.2 to the body length. The nuclei (1.6 × 2.5 μ) lie near the center of the disc.

The parabasal bodies are two rod-shaped organelles located near the sucker disc and lie at an angle with the axostyles.

Type slide: U. S. Nat. Mus. No. 9154.

DISCUSSION

Species of *Giardia* are distinguished largely on host specificity, dimensions, and shape of the organism, and the character of some chromophilic rods commonly referred to as the parabasal bodies. Whether or not these diagnostic characters are reliable will no doubt depend on the results of biological experiments and the study of variations in strains. Our present knowledge on these points is sadly in need of clarification.

Judging from the data at hand, and the method used by protozoologists to separate species of *Giardia*, the species described in this paper are considered to be distinct. They are given specific names that may serve to associate the species with the host. Biological experiments may prove the variations to be due only to host differences or to strain variations.

TABLE 1.—*Measurements in microns of 10 specimens of* Giardia ardeae, G. floridae *n. sp.*, G. botauri *n. sp.*, G. melospizae *n. sp.* G. sturnellae *n. sp.*, G. ondatrae *n. sp. and* G. microti. *The numbers enclosed in parenthesis are those obtained from measurements of 100 specimens.*

	G. ardeae after Hegner (1925)	*G. floridae* n. sp.	*G. botauri* n. sp.	*G. melospizae* n. sp.	*G. sturnellae* n. sp.	*G. ondatrae* n. sp.	*G. microti* after Hegner (1925)
Length of body	11.2	9.7(9.1)	11.2(10.4)	8.8(9.0)	9.8(9.1)	11.8(11.7)	11.8
Width of body	7.8	5.7(5.7)	7.0 (6.6)	5.7(6.0)	5.7(5.7)	6.3 (6.4)	6.6
Anterior end to center of nucleus	3.9	3.4	4.0	3.0	3.3	3.5	3.4
Center of nucleus to ends of lateral shields	5.3	3.3	4.0	3.5	3.2	4.1	4.7
End of lateral shields to posterior end	2.4	3.0	3.2	2.3	3.3	4.2	3.9
Width across lateral shields	2.4	2.8	2.7	3.1	3.4	3.0	3.8
Ratio of length to width	1.4	1.6	1.6	1.5	1.6	1.8	1.7
Length of sucker disc	7.1	5.3	6.0	4.9	4.5	6.1	5.3
Ratio of length to length of sucker disc	1.6	1.7	1.7	1.8	2.0	1.9	2.2

The writer is indebted to Dr. Logan J. Bennett of Iowa State College for allowing the study of parasites from one American bittern, one swamp sparrow, and two little blue herons, collected by him.

SUMMARY

Five new species of *Giardia* are described, four from birds and one from a mammal. *Giardia botauri* n. sp., size 11.2×7.0 μ, from the American bittern, *Botaurus lentiginosus* (Montagu), differs in dimensions from other species of giardia from the heron and bittern groups; *G. floridae* n. sp., size 9.7×5.7 μ, from the little blue heron, *Florida caerulea caerulea* (L.), differs in dimensions and shape of the parabasal body from giardia from the heron and bittern groups; *G. sturnellae* n. sp., size 9.8×5.7 μ, from the western meadowlark, *Sturnella neglecta* (Aud.), which is similar to *G. muris*, but differs in dimensions from the rodent species; *G. melospizae* n. sp., size 8.8×5.7 μ, from the swamp sparrow, *Melospiza georgiana* Latham; *G. ondatrae* n. sp., size 11.8×6.3 μ, from the muskrat, *Ondatra zibethicus* (L.), which is similar to *G. microti*, but differs in dimensions from the latter species.

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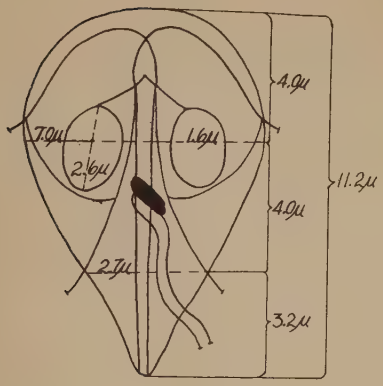
EXPLANATION OF PLATE

These figures are based on measurements of 10 specimens of each species.

FIG. 1. *Giardia botauri* n. sp. from the American bittern.
FIG. 2. Cyst of *G. botauri*.
FIG. 3. *Giardia floridae* n. sp. from the little blue heron.
FIG. 4. *Giardia melospizae* n. sp. from the swamp sparrow.
FIG. 5. *Giardia sturnellae* n. sp. from the western meadowlark.
FIG. 6. *Giardia ondatrae* n. sp. from the muskrat.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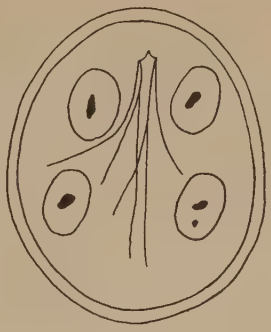

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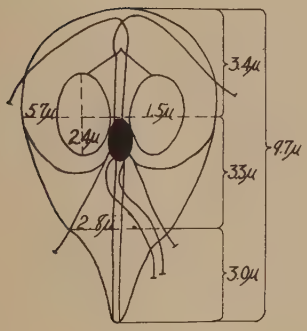
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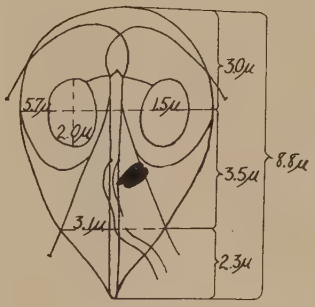
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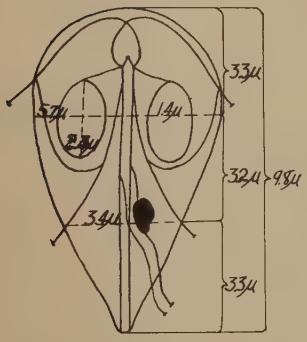
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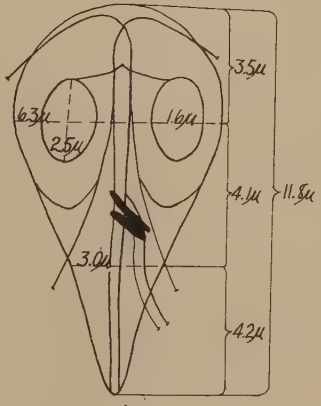
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A STUDY OF LARVAL TREMATODE INFECTIONS IN *HELISOMA CAMPANULATUM SMITHII* (BAKER) IN THE DOUGLAS LAKE REGION, MICHIGAN*

W. W. Cort, D. B. McMullen, and Sterling Brackett

Data from collections of *H. campanulatum smithii* (Table 1) from two areas, one extending for half a mile along the shore of the southeast corner of Twin Lakes and the other covering about one-fourth of a mile of the shore of a small nearby lake (Bullfrog Lake), gave information which supplemented an earlier study of the ecological relations of larval trematodes (Cort, McMullen, and Brackett, 1937). The trematode infections found in these snails included five species of plagiorchiid cercariae, one schistosome, a strigeid, an unidentified clinostome, and an unidentified amphistome. The life cycles of three of the plagiorchiids are known: *Macroderoides typicus* (McMullen, 1935), *Alloglossidium corti* (McCoy, 1928; McMullen, 1935; Crawford, 1937), *Cercorchis medius* (McMullen, 1934). The two others which are undescribed are given their laboratory names. The schistosome was identified as *C. wardi* (Miller, 1926) and the strigeid as the cercaria of *Crassiphiala amblophtis* (Krull, 1932; Hunter, 1935).

The snails collected were only of the largest size. In the Twin Lakes area, the snails of this group died in large numbers between June 18 and August 2, 1937, since numerous empty shells and those still containing dead bodies were found. While death may have been due to the completion of their life span, the extremely heavy infections may also have been a factor.

Except for the two commonest plagiorchiids, the number of positives of the species of cercariae was so small that differences in their incidence in the collections may have been due to chance rather than to seasonal fluctuations (Table 1). The high incidence of the cercaria of *M. typicus* in the Twin Lakes area and of the cercaria of *A. corti* in the Bullfrog Lake area is very striking. The first of these species shows a uniformly high incidence and the second gives some indication of an increase during the summer. These species must have been carried over the winter in large numbers in the snails, since it seems impossible that the large numbers of infections in the June 18th collections could have been acquired the same season. The small number of immature positives which was found is not surprising since it is almost impossible to identify immature

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* A contribution from the University of Michigan Biological Station and the Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University.

TABLE 1.—*Larval trematode infection in* Helisoma campanulatum smithii *from two areas in the Douglas Lake region*

	Twin Lakes Area				Bullfrog Lake Area			
	7/31/36	6/18/37	8/2/37	8/26/37	7/31/36	6/18/37	8/2/37	8/26/37
Total number of snails in collection	169	246	124	134	99	132	161	112
Number of immature (unidentified) positives	0	1	0	1	1	7	0	0
Percentage of negatives	1.2	5.3	1.6	8.2	9.1	42.4	15.5	13.4
Percentage incidence of species of cercariae:								
Plagiorchiids								
Cercaria of *Macroderoides typicus*	91.1	84.9	66.1	62.4	2.0	0	0	0
Cercaria of *Alloglossidium corti*	18.3	14.7	49.2	27.1	68.4	45.6	71.4	67.9
Cercaria of *Cercorchis medius*	1.2	0	0	0	2.0	4.0	1.2	1.8
Stylet M	0.6	0	0	0	5.1	3.2	0.6	0
Stylet Z	0	0	0	0	0	0	5.0	4.5
Schistosomes								
Cercaria wardi	1.2	0.4	1.6	1.5	8.2	0.8	6.8	8.9
Strigeids								
Cercaria of *Crassiphiala ambloplitis*	0	0.4	0.8	5.3	1.0	0.8	0.6	0
Clinostomes								
Cercaria of *Clinostomum* sp.	0	0	0	0	7.1	3.2	3.1	7.1
Amphistomes								
Amphistome cercaria (unidentified)	0	1.2	0	3.0	0	0	0.6	2.7

infections, when they are superimposed on mature ones. In the June 18th collection from the Bullfrog Lake area all of the 7 immature positives were plagiorchiids, probably *A. corti,* since this species showed a considerable increase in the next collection. The amount of infection of the intermediate host by these two fish trematodes indicates the very closest relationship between their hosts. It is conceivable that these heavy infections have a profound influence on the life cycle of this species of snail in the areas studied and perhaps retard the development of other larval trematodes.

Table 2 lists the multiple infections in these collections. An analysis

TABLE 2.—*Record of multiple infections in the collections of table 1 in relation to their occurrence by chance*

Combinations in multiple infections in total collection	From Twin Lakes Area		From Bullfrog Lake Area	
	No.*	Ch.*	No.	Ch.
Cer. *M. typicus* + Cer. *A. corti*	56	121	0	1
Cer. *M. typicus* + Cer. *C. medius*	2	2	0	<1
Cer. *M. typicus* + Stylet M	1	<1	0	<1
Cer. *A. corti* + Cer. *C. medius*	0	<1	2	5
Cer. *A. corti* + Stylet M	0	<1	3	6
Cer. *A. corti* + Stylet Z	0	0	4	9
Cer. *C. medius* + Stylet Z	0	0	1	1
Cer. *M. typicus* + Cer. *wardi*	1	5	0	<1
Cer. *A. corti* + Cer. *wardi*	2	2	10	21
Cer. *C. medius* + Cer. *wardi*	0	<1	1	2
Cer. *M. typicus* + Cer. *C. amblopliti*s	2	5	0	<1
Cer. *A. corti* + Cer. *C. amblopliti*s	0	3	0	2
Cer. *A. corti* + Cer. *Clinostomum* sp.	0	0	0	16
Cer. *M. typicus* + Amphistome cercaria	1	4	0	0
Cer. *A. corti* + Amphistome cercaria	0	2	0	3

* No. = actual number; ch. = number expected by chance.

by the method used in our earlier paper (*l.c.*) is given of the number of times the various combinations occurred in double infections as compared with what would have been expected by chance. Except possibly in the case of the combination of the cercaria of *Clinostomum* sp. with that of *A. corti* there is no significant evidence of any immunity or antagonism operating to prevent certain combinations.

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SURVIVAL AND LOCATION OF THE MICROFILARIAE OF *DIROFILARIA IMMITIS* IN THE DOG

PAUL C. UNDERWOOD* AND PAUL D. HARWOOD

Zoological Division, Bureau of Animal Industry,
U. S. Department of Agriculture

The length of life of the microfilariae of *Dirofilaria immitis* in the blood of dogs has become recently a subject of considerable interest, since one of the popular explanations of the periodicity of the microfilariae becomes invalid if the microfilariae normally live longer than a day or two. In an attempt to obtain information on the longevity of the larvae of *Dirofilaria immitis,* an uninfected dog was transfused with blood containing microfilariae of this parasite.

HISTORICAL

The transfer of blood containing living microfilariae from infected to uninfected animals, either of the same or of different species, has been reported many times in the literature. Gruby and Delafond (1852) and Fülleborn (1908, 1912) have reported that microfilariae of *Dirofilaria repens* have survived more than 2½ years in dogs uninfected with adult worms. The latter author reported growth of the microfilariae in the blood of the experimental dog, but observed no change suggesting the metamorphosis which ordinarily takes place in the mosquito intermediate host. Augustine and Drinker (1935) as well as Drinker, Augustine, and Leigh (1935) have injected microfilariae of *Dirofilaria immitis* into the blood or lymphatic system, respectively, of uninfected dogs, and have demonstrated that these larvae frequently leave the blood stream and appear in considerable numbers in the lymphatics. Hinman, Faust, and De Bakery (1934) have studied the periodicity of microfilariae of *D. immitis* after transfusion of infected blood into an uninfected dog. Murgatroyd (1933), Knott (1935) and Ray (1936) have transferred blood containing active microfilariae of *Wuchereria bancrofti* to human hosts who did not show microfilariae on blood examination at the time of transfer. Tisseuil (1935) has transferred infected blood to young opossums, *Philander* sp. The transfer of microfilariae to species of animals in which that parasite does not normally occur is a different type of experiment from that reported in this paper. Therefore, only the

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* The experiment was planned and all data were collected by the senior author, but owing to his transfer to the U. S. Bureau of Dairy Industry, October 15, 1937, it was impossible for him to prepare the data for publication. Therefore, the data were analysed and this paper was prepared for publication by the junior author.

literature dealing with the transfer of microfilariae to their normal but uninfected hosts is of interest in connection with the work reported in this paper.

MATERIALS AND METHODS

The donor, a bull terrier, was obtained from Miami, Florida, and at the time the transfusion was made the peripheral blood contained 2,332 microfilaria per cc. The recipient, a male Gordon setter, weighed 26.8 kilograms at the time of transfusion. This animal was obtained from the vicinity of Washington, D. C., in October, 1932, and was about four months old when received; from that time until used in the transfusion experiment he had been kept in the kennels of the Zoological Division at the National Agricultural Research Center, Beltsville, Maryland. During that time he remained negative for microfilariae.

During the course of the experiment, blood from the jugular vein of the recipient was examined at frequent intervals for microfilariae. At first only 1 cc quantities were withdrawn for examination, but as the microfilariae became fewer this was increased to 10 cc. The blood examined was hemolysed with 3 per cent acetic acid, centrifuged, the supernatant fluid poured off, and the sediment washed in several changes of the dilute acetic acid. The entire sediment was then examined for microfilariae. All samples of the blood were taken between 11 A M and 2 P M.

During the course of this experiment the recipient was kept in a cage in a room screened against mosquitoes, except when turned out for exercise during the day, in a yard where mosquitoes were not found. At autopsy a search was made for adult heartworms, and for lesions which would indicate that adult *Dirofilaria immitis* had been present at one time. Neither lesions nor heartworms were found. These precautions were possibly superfluous since dogs maintained with infected carriers in kennels unprotected from mosquitoes did not contract heartworm infection at Beltsville, Maryland.

EXPERIMENTAL DATA

The transfer of 100 cc of blood from the donor to the recipient was made on March 4, 1935. The recipient appeared to be unaffected by the treatment. According to calculations the recipient received 233,200 microfilariae along with the blood, and assuming that these microfilariae became evenly distributed throughout the circulation, there should have been approximately 100 microfilariae per cc of blood. On March 5, 1935, or one day after the transfusion, 1 cc of blood was withdrawn from the jugular vein of the recipient, but only 4 microfilariae were observed following centrifugation.

Subsequent counts of the microfilariae appearing in the peripheral blood stream of the recipient are given in table 1. This table does not

TABLE 1.—*Counts of microfilariae appearing in the peripheral circulation of a dog transfused March 4, 1935, with blood containing microfilariae of* Dirofilaria immitis

Date of count	Days since transfusion	Blood examined: cc	Number of microfilariae	
			Counted in sample	Calculated basis 10 cc blood
March 5, 1935.	1	1	4	40
March 6	2	1	8	80
March 7	3	1	7	70
March 8	4	1	4	40
March 11	7	1	1	10
March 12	8	1	7	70
March 13	9	1	7	70
March 14	10	1	5	50
March 15	11	1	5	50
March 18	14	1	8	80
April 13	40	5	2	4
May 7	64	1	3	30
June 5	93	1	3	30
July 1	119	1	2	20
August 5	154	5	12	24
September 5 ...	185	2	2	10
October 19	229	5	2	4
November 6 ...	247	5	8	16
December 9 ...	280	5	5	10
January 4, 1936	306	5	0	0
February 11 ...	344	5	3	6
March 5	366	5	0	0
April 3	395	5	3	6
May 8	430	5	3	6
June 5	458	5	4	8
July 2	485	5	7	14
August 18.....	532	5	2	4
September 16 ..	561	5	6	12
October 5	580	5	0	0
November 25 ..	631	10	2	2
December 30 ..	666	10	1	1
January 25, 1937	692	10	3	3
February 15 ...	713	10	3	3
March 4	730	10	4	4
May 13	800	10	1	1
July 1	849	10	18	18
July 21	869	10	6	6
September 27 ..	937	10	0	0
October 1	940	10	0	0

show all the observations made on this animal, but all necessary data for purposes of illustration are included. These data represent the first 10 observations made, and from the subsequent counts the first observation made in each month was selected. An exception was made of the date July 21, 1937, as this was the date of the last examination showing microfilariae in the blood samples. This system was followed in order to eliminate any selection of dates, either consciously or unconsciously, that might give to the data a more uniform appearance than was the case in the actual observations.

During the course of the experiment the recipient was treated once with normal butyl bromide for the removal of hookworms and with lime-sulphur dip once for the destruction of mange mites. Apparently, neither treatment affected adversely the survival of the microfilariae.

The microfilariae were last observed in the blood of the recipient on July 21, 1937, or about 2 years 4½ months after the date of transfusion. The dog was killed on October 6, 1937. At autopsy 10 cc samples each of blood from the heart and lungs were taken and examined for microfilariae with negative results. Examination showed 14 *Ancylostoma caninum* in the small intestine, but no other helminths.

At no time in the course of this experiment was any increase in the size of the microfilariae or any morphological change in the microfilariae noted.

COMPARISON OF DATA ON SURVIVAL OF MICROFILARIAE IN THEIR NORMAL HOSTS

The first experiment to test the survival of microfilariae in the blood of dogs was performed by Gruby and Delafond (1852). The experimental dog was still infected when he died from causes other than filariasis about three years later. However, puppies kept by these investigators became infected naturally when only 6 months old, and it is possible that the dog used in the microfilarial survival experiment also became infected naturally with adult worms during the course of the investigation. Nothing was known at that time of the role of the mosquito in the transmission of filarids, so these investigators probably took no precautions to prevent their experimentally infected animal from attack by these insects. Apparently Gruby and Delafond worked with *Dirofilaria acutiuscula* (according to Chitwood, *D. repens* is a synonym of *D. acutiuscula*).

Fülleborn (1908, 1912) was able to demonstrate the microfilariae of *Dirofilaria acutiuscula* in the blood of a dog more than 2 years after the injection of infected blood. At autopsy, more than 2 years and 9 months after the date of infection, microfilariae were still found to be numerous in blood from the lungs. These larvae were more than twice their original size. Except for the lack of increase in size of the embryos, the results of the present experiment with *Dirofilaria immitis* are in very close agreement with those of Fülleborn. Therefore, it is apparent that the microfilariae of *Dirofilaria* species may live for two or more years in the blood stream of the dog.

It is possible that in dogs which have become heavily infected with adult worms and microfilariae, the latter may occasionally be destroyed either through the action of specific antibodies, or through other immunological mechanisms in a much shorter period than two years, but regardless of such possibilities the fact remains that microfilariae do survive for many months under optimum conditions in their natural definitive hosts, and the work of Hinman (1935) suggests that the microfilariae of *Dirofilaria immitis* are normally long lived. Hinman counted the micro-

filariae in the peripheral blood, then killed the host and examined it post mortem for adult worms. By assuming that the blood of a dog equals 1/12 of the body weight, and that the microfilariae are evenly distributed in the blood stream, Hinman calculated the number of microfilariae present per female worm. If the microfilariae live normally for two years it is to be expected that this method of calculation would yield highly variable results, such as Hinman obtained. The writer does not mean to infer that this is the only possible explanation of the variation which Hinman obtained, but that his observations are in keeping with expectations, if the microfilariae live normally for two years.

Hinman calculated that the microfilarial population in 30 dogs varied from 10,740 to 3,750,000 microfilariae per female worm. The latter figure certainly suggests that the microfilariae had been accumulating for some time in that host. Yet, as will be shown later in this paper, Hinman's method of calculation will usually include only one-twentieth of the microfilarial population actually present in the host. Therefore, it is probable that microfilariae frequently live for many months under normal conditions, whether the host is infected with adult worms or not.

Information on the longevity of other species of microfilariae is somewhat meager. Knott's (1935) experiments indicate that microfilariae of *Wuchereria bancrofti* may live for two or more weeks in their normal host. However, as the subject used in the experiment reported by Knott may have been naturally infected, the validity of his experiment is open to question. Fülleborn (1929) injected microfilariae of *W. bancrofti* into the thoracic cavity of a mouse and was able to demonstrate the larvae in that location 10 days later. In view of these reports by Knott and Fülleborn, it seems probable that the microfilariae of *W. bancrofti* may live normally for 10 days or more.

Microfilariae of *Dipetalonema perstans* were found to persist in the blood stream of a dog for two months and in the pulmonary circulation of a mouse for 67 days (Fülleborn, 1929). As both the dog and mouse are only experimental hosts for this parasite, the value of these data in estimating the normal length of life of the microfilariae in man is debatable. However, since animals usually live longest in their normal environment, one might expect that in man these parasites would be able to survive at least as long as in the dog and the mouse. Tisseuil (1935) demonstrated that microfilariae of an unnamed filarid parasite of *Philander* sp. could live in the blood stream of that host for 6 to 11 days, but not for as long as 3 weeks. Although it seems evident that the microfilariae of various species of filarids survive for extremely variable periods in their normal hosts, data reported heretofore have contributed little to invalidate and much to substantiate Fülleborn's (1929) conclusion that "Ganz sicher ist der Annahme von Myers, dass die Mikrofilarien des Menschen nur wenige Stunden lebten, falsch; . . ."

MICROFILARIAE AND THE MACROPHAGE SYSTEM

Lane (1937a) has calculated that more than 90 per cent of the 3 million microfilariae which Hinman, Faust, and De Bakery (1934) injected intravenously into a dog were at once removed by the cells of the reticulo-endothelial system and subsequently destroyed. At first glance, the proven longevity of microfilariae seems to invalidate this theory, but it may be advisable to examine the theory further.

To establish his theory, Lane has assumed that the microfilariae are evenly distributed in the blood stream, and that the samples which Hinman *et al.* obtained represented a fair sample from the entire living microfilarial population in their experimental animal. Accordingly, the capacity of the reticulo-endothelial system of a 12-pound dog for destroying these parasites would be fixed at about three million. In a larger dog this capacity should be relatively greater. Therefore, to determine whether or not Lane's theory is tenable, a comparison of the data submitted by Fülleborn (1908), by Hinman, Faust, and De Bakery (1934), by the present writers for *Dirofilaria* species, and by Knott (1935) for *Wuchereria bancrofti* is given in table 2. Hinman *et al.* (1934) and Lane (1937a) have assumed that the blood in an animal approximates 1/12 of the total body weight; for purposes of calculation that figure is used in this paper. As some selection of data has been necessary, the writers have used in each case an average of several figures which were obtained shortly after the date of infection.

As shown in table 2 between 4.2 per cent and 6 per cent of the microfilariae of *Dirofilaria* species injected into a dog can be accounted for if one assumes that the microfilariae are evenly distributed in the blood stream of the host. Considering the inaccuracies of the methods used, this is a surprisingly close agreement in the percentage of recovery of microfilariae. However, since Hinman *et al.* injected 707 microfilariae per gram of body weight, whereas the writers injected only 9, these figures give no evidence of destruction of a constant number of microfilariae, but indicate that usually only a small percentage of injected microfilariae appear in the peripheral circulation and that the majority seek other habitats.

If the results of Knott's (1935) experiment (case E. D.) are compared with the experiments on dogs with *Dirofilaria* species, it appears that the behavior of microfilariae in their respective hosts is similar, but in the human infection with *Wuchereria bancrofti* a somewhat greater percentage of the microfilariae may circulate in the peripheral blood.

The morphological evidence for the rapid destruction of microfilariae cited by Lane (1937a and b) may possibly be explained on the basis of an acquired immunity which has activated the host's macrophage system. Taliaferro (1934) has pointed out the great similarity existing between

TABLE 2.—*Relationship between the number of microfilariae injected and the number subsequently found in the peripheral circulation*

Parasite	Name of host	Weight of host (kg)	Number of microfilariae injected per gram of body weight	Percent of injected microfilariae found subsequently, assuming even distribution in bloodstream	Authority
Dirofilaria acutiuscula	dog	2.2	213	6	Fülleborn (1908)
Dirofilaria immitis	dog	5.45	707	4.2	Hinman *et al.* (1934)
Dirofilaria immitis	dog	26.8	9	5.8	Underwood and Harwood (present paper)
Wuchereria bancrofti	man	70*	19.2	10	Knott (1935; case E.D.)

* Estimated.

resistance as manifested against *Wuchereria bancrofti* (O'Connor and Hulse, 1932; O'Connor, 1932) and resistance as manifested against pathogenic organisms of other types. Apparently the macrophage system is involved in the destruction of filariae, but the long period of survival of the microfilariae in the normal host indicates that the destruction of microfilariae by this system is very slow until it has been activated by a specific antibody. Skin tests have demonstrated the existence of such immune bodies in cases of filariasis.

Lane's theory (1937a) does not require activation of the macrophage system by an acquired immunity, since the theory as presented states that this system attacked in a short time the majority of the microfilariae which Hinman *et al.* (1934) injected into a normal dog. However, the original theory of Myers referred to by Lane deals with the presence of microfilariae in a host infected with adult worms, and it may be assumed that the growth products of these adults in some manner activate the macrophage system, that under normal conditions rapid destruction of microfilariae is the rule, and that this destruction of microfilariae, coupled with cyclical simultaneous parturition, is sufficient to explain the mechanism of microfilarial periodicity. But it is generally admitted that microfilarial periodicity is a remarkably constant phenomenon, whether studied simultaneously in several individuals from one area, or whether studied at different times in the same individual. A change in the habits of the host is the only factor which markedly disturbs the rhythm of microfilarial periodicity. On the other hand, it is well known that antibody formation differs markedly in different individuals, and it must be expected that any phenomenon which is dependent on antibody formation or other types of acquired immunity for its activation will be highly variable. The sudden and more or less permanent disappearance of microfilariae from the blood stream of the infected host, the onset of filarial fever and of elephantiasis are unpredictable phenomena which may be expressions of the various types of immunity, but it seems unlikely that any phenomenon which is relatively as regular in its appearance as microfilarial periodicity could be dependent on an immunological activation of the macrophage system.

LOCATION OF THE MICROFILARIAE IN THE BODY OF THE HOST

If the microfilariae are unevenly distributed in the blood stream it may be expected that at some time they will increase suddenly in the peripheral circulation. In Fülleborn's experiment (1908, 1912) this is precisely what happened. From data collected 10 months after infection it was calculated, on the basis of an even distribution, that the blood stream contained approximately 300,000 microfilariae or more than one-half of the total injected and ten times that found in previous examina-

tions. Obviously all these microfilariae must have been in the body during the 10 months which had elapsed from the time of infection, since no adult worms were present as Fülleborn (1912) subsequently ascertained.

At this time we cannot be certain of the location of the main mass of the microfilariae, but it seems reasonable to expect that in spite of their activity the parasites are held back in the capillaries throughout the body. If this be true, the smaller microfilariae should be able to pass the capillary network faster and more easily than the larger forms. This seems to be suggested by the findings of Fülleborn (1929) who injected 250,000 microfilariae of *Loa loa* and 20,000 microfilariae of *Dipetalonema perstans* into the blood stream of a dog, and subsequently was able to find only three larvae of *Loa loa* although numerous larvae of *D. perstans* were present in the blood stream for more than a month. As it is common knowledge that capillary size is reflected closely by the size of the erythrocytes, the latter being smaller in a dog than in man, it appears possible that the large microfilariae of *Loa loa* (301 μ by 7 μ) were unable to pass the capillary network in the dog used by Fülleborn, although the same capillaries were easily passed by microfilariae of *Dipetalonema perstans* (199 μ by 3 to 4 μ). When microfilariae of *Wuchereria bancrofti* are injected into the blood stream of a guinea pig they promptly disappear, a phenomenon which may be due to the small bore of the capillaries in this animal. For these, and other reasons, Fülleborn (1929) concluded that the main mass of the microfilariae were to be found in the capillary network.

Drinker, Augustine, and Leigh (1935) as well as Augustine and Drinker (1935) have shown that not only are the microfilariae of *D. immitis* able to burrow in the tissues, but that they frequently do this. Furthermore, it has been demonstrated by O'Connor (1931) that the sheathed microfilariae of *Wuchereria bancrofti* and by Harwood (1932) that those of the closely related *Litomosoides pattersoni* can burrow in the tissues without loss of, or damage to, their sheaths. Therefore, it is probable that microfilariae injected into the blood stream do not remain confined to that system, but wander into the lymph spaces and the lymphatic system in every part of the body. These observations support Fülleborn's (1929) suggestion: "dass *vielleicht alle* Mikrofilarienarten gelentlich aus den Blutbahn in die Lymphspalten austreten können."

SURVIVAL OF MICROFILARIAE AND CYCLICAL PARTURITION OF THE FEMALES

The demonstrable length of life of microfilariae in the vertebrate host indicates that periodic simultaneous parturition of the female worms and a correlated rapid destruction of the microfilariae attaining the blood

stream by a non-activated macrophage system do not constitute an adequate explanation for the periodic appearance of microfilariae in the peripheral circulation. The data cited above do not prove or disprove the existence of cyclical simultaneous parturition in the female worms of any species of filarid, but they do show that the phenomenon above will not explain adequately the manifestations of microfilarial periodicity, unless some type of immunological reaction destroys the microfilariae much more rapidly than they are destroyed in a host that is not infected by adult worms. For various reasons, which are briefly discussed above, the presence of such a reaction seems unlikely.

SUMMARY

1. An uninfected dog was transfused intravenously with blood containing approximately 233,000 microfilariae of *Dirofilaria immitis*.

2. The microfilariae survived in the blood stream of this dog for more than two years.

3. At autopsy, about 2½ years after the injection of the microfilariae, no embryos could be found in the blood.

4. No increase in size of the embryos was noted at any time.

5. As other authors have noted, comparatively few of the introduced microfilariae appeared in the peripheral circulation following injection.

6. Lane's hypothesis that most of the artificially introduced microfilariae are removed by the cells of the reticulo-endothelial system and subsequently destroyed, is not substantiated.

7. Available evidence indicates that a part of the microfilariae are concentrated in the capillary networks throughout the body, and a part leave the blood stream altogether; this is possibly true for all microfilariae in the blood stream, whether sheathed or unsheathed.

8. Whether or not the microfilariae live for long periods under natural conditions in the presence of adult worms can not be ascertained on the basis of available data. There is evidence that the macrophage system, activated by some type of immunological reaction, may destroy large numbers of microfilariae in a short time, but there is also evidence which suggests that such reactions are of irregular occurrence, and that they do not regularly affect microfilarial longevity or periodicity.

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NOTES ON THREE NEW SPECIES OF CERCARIAE BELONGING TO THE PLEUROLOPHO-CERCA GROUP

R. M. Cable and N. C. Wheeler
Department of Biology, Purdue University, Lafayette, Ind.

An extended study of the trematode parasites of *Goniobasis semicarinata* (Say) has resulted in the discovery of three new species of cercariae belonging to the Pleurolophocerca group as outlined by Sewell (1922), and emended by Vogel (1934) and Cable (1938). Since the latter paper also gave a summary of the described species and known relationships of pleurolophocercous cercariae, this information is not included in the present report.

At intervals during the past two years, specimens of *G. semicarinata* have been collected in large numbers from streams in Madison County, Kentucky, and brought to Lafayette for observation. The technique used in the present study was the same as that given by Cable (1938). All measurements below were taken from living material under light cover glass pressure and represent averages of several determinations. All drawings were made to scale from numerous observations and measurements of structures represented.

It was noted that behavior was of great value in differentiating the species found; in fact, differences in behavior of the cercariae first suggested that species other than *Cercaria vogeli* were present, and confirmatory morphological studies were made. Striking differences in behavior of morphologically similar species were noted, and also similarities of behavior were observed in species differing greatly in certain respects, particularly size. The junior author has undertaken a comparative study of the behavior of the species found and the results of this investigation will be reported at a later date.

Cercaria opacocorpa n. sp.

(Figs. 1, 5, 8, 9, 10 and 11)

Specific diagnosis: Small pleurolophocercous cercaria with the characteristics of the group. Photopositive and sensitive to shadow stimuli; intermittent swimmer, average duration of swimming movement 0.8 sec. Eye-spots medium in size and consisting of cuboidal masses of pigment granules; body heavily pigmented, averaging 0.16 mm in length by 0.07 mm in width. Spines prominent on anterior end of body, but difficult to observe posterior to eye-spots. Tail averages 0.4 mm in length and 0.024 mm in maximum width. Caudal fin-fold begins dorsally near base of tail and terminates ventrally at a distance from posterior end of tail equal to about one-third length of tail. Cuticula of base of tail distinctly elevated on ventral side only. Oral sucker elongate, protrusible, and measuring 0.04 mm in length by 0.03 mm in width. Small acetabulum present. Prepharynx

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slightly longer than pharynx; pharynx 0.012 mm long and 0.01 mm wide. Seven pairs of cephalic glands with ducts separated anteriorly to form two median bundles of four ducts each and two lateral of three each, all opening dorsal to mouth. Cystogenous glands scattered thruout body. Genital primordium just dorsal to acetabulum and consisting of mass of small nuclei. Excretory bladder medium in size and U-shaped. Intestinal ceca not observed. Cercariae develop in digestive gland of the mollusk in rediae similar to those of other pleurolophocercous species.

Host: Goniobasis semicarinata (Say)
Locality: Cowbell Creek, Madison County, Ky.

C. opacocorpa is very active when mounted for study and the body is very changeable in shape. It may be extended to a length of over 0.26 mm and contracted until less than 0.11 mm. The tail may be extended to a length of 0.46 mm and its distal end is especially contractile as in the other species studied.

The body is densely pigmented posterior to the oral sucker. Most of the pigment occurs in the superficial tissues. On the ventral side of the body, two non-pigmented areas appear as longitudinal bands which converge posteriorly (Fig. 9). The eye-spots are medium in size, measuring about 0.007 mm in width. Double eye-spots have not been observed in this species. On the ventral side of the proximal part of the tail, the cuticula is elevated 0.007 mm for a distance of about 0.07 mm from the posterior end of the body (Fig. 5). On the dorsal side, the fin-fold attains a maximum width of 0.022 mm and narrows to a width of 0.006 mm before passing around the tip of the tail. It widens abruptly on the ventral side, attaining a maximum width of 0.016 mm. The posterolateral parts of the body on either side of the tail socket are somewhat pointed in this species and also in *C. semicarinatae*. Dorsally, the posterior end of the body is rather evenly rounded, or very slightly incurved, and overlies the base of the tail, while on the ventral side, the posterior end of the body is deeply grooved to form the tail socket. After decaudation, the pronounced ventral flexure of the posterior end of the body persists, so that the posteroventral body wall is thrown into transverse folds.

The anterior end of a typical pleurolophocercous cercaria is exemplified by that of *C. opacocorpa*. The position of the oral sucker when retracted, the spines on its dorsal lip, and the openings of the cephalic gland ducts are shown in ventral view in Fig. 8, and in lateral aspect in Fig. 10. The relationship of these structures when the oral sucker is protruded is shown in Fig. 11.

Although the entire body is covered with spines, they are much smaller and more difficult to observe posterior to the pharynx. Imbricated rows of minute spines may be seen by using rather strong illumination and focusing with the oil immersion objective until the surface of the body is barely in focus.

C. semicarinatae n. sp.

(Figs. 2 and 6)

Specific diagnosis: Small pleurolophocercous cercaria with the characteristics of the group. Not phototactic; swims almost continuously with extremely short rest periods (usually less than 1 sec.). Eye-spots large, cuboidal masses of pigment granules; very light body pigmentation. Body averages 0.15 mm long and 0.08 mm wide. Prominent spines on anterior end of body; spines small and difficult to observe posterior to eye-spots. Tail averages 0.36 mm in length and 0.03 mm in maximum width. Caudal fin-fold begins dorsally about one-third length of tail from posterior end of body and terminates ventrally a distance from posterior end of tail equal to about two-fifths length of tail. Cuticula of base of tail not distinctly annulated or elevated laterally, but is slightly raised ventrally. Oral sucker 0.025 mm long and about 0.03 mm wide, not as protrusible as in other species; mouth cavity small; pharynx not observed; intestinal ceca undeveloped. Small acetabulum present. Seven pairs of cephalic glands in mid-dorsal body region; ducts separated anteriorly to form two median bundles of four each and two lateral of three each, all opening dorsal to mouth; cystogenous glands scattered throughout body. Genital primordium a mass of small nuclei immediately anterior to excretory bladder and dorsal to acetabulum. Excretory bladder medium in size and distinctly U-shaped. Cercariae develop in digestive gland of the mollusk in simple, elongate rediae similar to those of other pleurolophocercous cercariae.

Host: Goniobasis semicarinata (Say)

Locality: Cowbell Creek, Madison County, Ky.

When studied alive, *C. semicarinatae* is very active under the cover glass. The body may be extended to a length of over 0.2 mm and contracted to half this length. When extended, the oral sucker is spherical, measuring 0.035 mm in diameter. The tail may be extended to nearly 0.4 mm in length; its tip is especially contractile and, when shortened, throws the distal part of the fin-fold into numerous plicae.

C. semicarinatae is less pigmented than any of the other species studied. The pigment that is present is distributed through the superficial tissues of the body in small granular masses. In the posterior half of the body, these masses are arranged in indistinct longitudinal rows. In comparison to the size of the body, the eye-spots are larger in *C. semicarinatae* than in any other species studied. They are cuboidal masses of dark pigment granules and measure about 0.01 mm in width. Double eye-spots as in *C. vogeli* and *C. constrictovesica* were not observed.

The cuticula of the tail is elevated only on the ventral side where it is much less distinctly raised than in *C. opacocorpa*. The fin-fold attains a maximum width of 0.017 mm on the dorsal side, narrows to about 0.007 mm before it passes around the distal tip of the tail, and again widens to a maximum of 0.012 mm on the ventral side.

The excretory bladder of *C. semicarinatae* resembles that of *C. opacocorpa* so closely that this structure is of no importance in differentiating these species. It maintains its U-shape even when dilated with excretory fluid. The excretory pore opens in the tail socket.

The nature and shape of the oral sucker serve to distinguish *C. semicarinatae* from other species. When the cercaria is at rest, the oral sucker (Fig. 6) is not withdrawn into the body as in other species (Fig. 10), but both the oral edge of the sucker and the openings of the penetration glands are exposed. When the body contracts, the sucker merely shortens and the mouth opening appears somewhat papillose. The cavity of the oral sucker is very narrow. A pharynx was not observed in this species although the adult stage is probably pharyngeate.

The distinguishing characteristics of *C. semicarinatae* are its behavior, the extent of the caudal fin-fold, light pigmentation of the body, and the nature of the oral sucker.

Cercaria constrictovesica n. sp.

(Fig. 3)

Specific diagnosis: Pleurolophocercous cercaria with the characters of the group. Photopositive and sensitive to shadow stimuli. Swims intermittently, frequently with characteristic halt in swimming movement. Average duration of swimming movement 1.5 sec. Left eye-spot frequently double in form; body moderately pigmented, averaging 0.16 mm in length by 0.12 mm in width. Anterior end with spines which seem to fade out posterior to eye-spots. Tail 0.44 mm long and 0.04 mm in maximum width minus cuticula; fin-fold begins dorsally about one-fourth length of tail from posterior end of body and terminates ventrally near middle of tail. Cuticula of proximal part of tail distinctly elevated and annulated. Oral sucker 0.04 mm in diameter; prepharynx short, pharynx 0.012 mm in diameter; esophagus and intestinal ceca undeveloped. Small acetabulum present. Seven pairs of cephalic glands with four bundles of ducts, two median bundles of four each and two lateral of three each, all opening dorsal to mouth. Genital primordium a mass of nuclei immediately anterior to excretory bladder and dorsal to acetabulum. Excretory bladder large, conspicuous, with pair of characteristic lateral constrictions. Cercariae develop in simple, elongate rediae characteristic of the Pleurolophocerca group.

Host: Goniobasis semicarinatae (Say)

Locality: Cowbell and Scaffold Cane Creeks, Madison County, Ky.

When studied alive, *C. constrictovesica* tends to lie quietly under the cover glass with the body rather strongly contracted. Specimens sometimes remain quiescent long enough to be drawn with the aid of the camera lucida. The body may exceed 0.2 mm in length when extended and may contract to a length of less than 0.13 mm. The single, right eye-spot averages about 0.008 mm in diameter, the left one about 0.011 mm in width, when double. Double eye-spots are frequently but not always observed. Their double nature seems to be more evident when the specimens are under considerable cover glass pressure. This observation suggests the possibility that in this species and in *C. vogeli* the double eye-spot may result from the disintegration of a single structure. If this were the case, however, it would be difficult to account for the fact that it is always the left eye-spot which appears to be double.

The cuticula of the base of the tail is distinctly raised and annulated for a distance of about 0.1 mm. The maximum elevation is about 0.006

mm and the width of the annulations averages about 0.005 mm. The dorsal part of the caudal fin-fold widens rapidly from its beginning to a maximum of about 0.025 mm, but as it approaches the distal end of the tail, it narrows to about 0.009 mm, retaining approximately that width as it passes around the tip of the tail. On the ventral side, the fin-fold attains a maximum width of approximately 0.012 mm and extends farther toward the base of the tail than in either *C. opacocorpa* or *C. semicarinatae.*

The excretory bladder of *C. constrictovesica* is characteristic as far as the species studied are concerned. It is much larger than in the others and, even when fully distended, shows a distinct transverse constriction. Less conspicuous secondary folds of the wall are sometimes observed. As in other species, the excretory pore opens into the tail socket. The posterolateral regions of the body on either side of the tail socket are not as pointed as in *C. semicarinatae* and *C. opacocorpa,* but are broadly rounded.

Cercaria vogeli Cable, 1935
(Figs. 4 and 7)

C. vogeli was originally described from *Goniobasis semicarinata* collected in Kentucky, but it has been found to occur also in *G. depygis* (Say) from McCormick's Creek, Owen County, Indiana.

Recent observations permit certain corrections and additions to Cable's (1938) description of this species. It has been noted that the ventral part of the caudal fin-fold does not terminate abruptly, as previously described, but becomes very narrow and continues proximally almost to the body, as shown in Fig. 4. The separation of the cuticula of the tail from the underlying tissues when mounted for study, together with the natural elevation of the cuticula near the body, almost obscure the very narrow continuation of the ventral fin-fold except in very favorable material. A rudimentary acetabulum has also been observed in *C. vogeli.* It is 0.025 mm in diameter and lies considerably anterior to the genital primordium, whereas in the smaller species studied, the acetabulum is ventral to the primordium. The acetabulum may be observed in other forms by focusing on the ventral surface of the body, but in *C. vogeli* there is only a very slight external fold, the primordium of the sucker being embedded in the body and obscured by the penetration glands. The number and distribution of the spines on the dorsal lip of the oral sucker are characteristic for this species (Fig. 7). They are arranged in three imbricated transverse rows, the two dorsal ones with about 14 spines each, the ventral with seven. In Fig. 7, the ducts of the penetration glands are shown in optical section.

DISCUSSION

The results of the present study indicate that it would be impossible to identify positively many described species of pleurolophocercous cercariae on the basis of existing descriptions. The number of cephalic glands and the arrangement of their ducts cannot be regarded as specific characters since a number of both freshwater and marine species seem to be practically identical in these respects. The precise nature of the tail, shape of the excretory vesicle, and the behavior of the cercariae are of great importance in distinguishing pleurolophocercous species. In all cases, the living cercariae must be studied. Although stained, whole mounts are helpful in observing the nature of the genital primordium, and sectioned specimens aid in determining the exact extent of body spination and the number of cephalic glands in heavily pigmented species, this material would be practically worthless in distinguishing between two species such as *C. opacocorpa* and *C. semicarinatae*. However, one unfamiliar with the material would notice immediately the striking difference in the behavior of these species and a critical study of their structure reveals certain slight but constant morphological differences. These observations are in agreement with those of Rothschild (1938) who has reported finding in *Perinoia ulvae* at least seven species of pleuro- and parapleurolophocercous cercariae, certain of which were difficult to differentiate "unless living material was available for comparison." Rothschild states that Lebour probably described these forms under one specific name, which is not surprising in view of the morphological similarity of certain species of pleurolophocercous cercariae.

The excretory pattern may be of specific value. However, it is so obscured by glandular structures and pigment masses in the majority of pleurolophocercous species that only a few of the flame cells and their capillaries can be observed.

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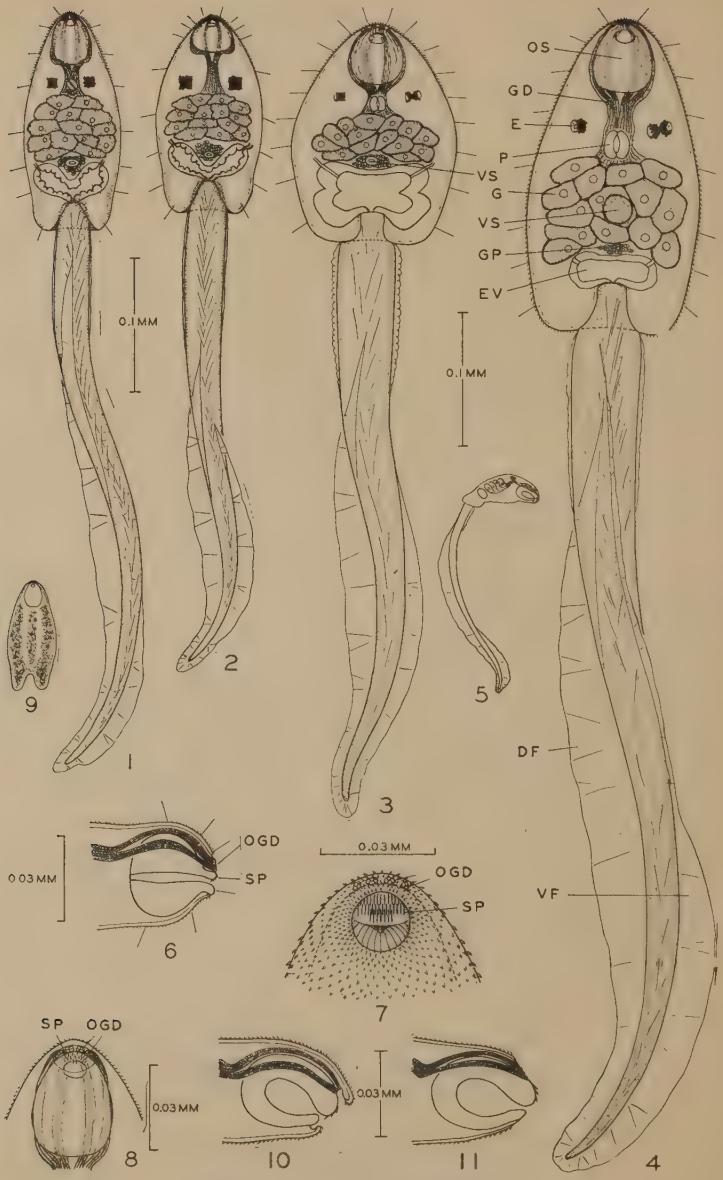
Explanation of Plate, p. 42

ABBREVIATIONS

df—dorsal fin-fold of tail
e—eye-spot
ev—excretory vesicle
g—cephalic glands
gd—ducts of cephalic glands
gp—genital primordium
ogd—openings of cephalic gland ducts
os—oral sucker
p—pharynx
sp—spines on dorsal lip of oral sucker
vf—ventral fin-fold of tail
vs—ventral sucker or acetabulum

Fig. 1. *Cercaria opacocorpa,* ventral view.
Fig. 2. *C. semicarinatae,* ventral view.
Fig. 3. *C. constrictovesica,* ventral view.
Fig. 4. *C. vogeli,* ventral view.
Fig. 5. *C. opacocorpa,* side view.
Fig. 6. *C. semicarinatae,* side view of anterior end, showing the appearance of the oral sucker when the body is contracted.
Fig. 7. *C. vogeli,* ventral view of anterior end, showing the characteristic arrangement of spines on the dorsal lip of the oral sucker. The ducts of the cephalic glands are shown in optical section.
Fig. 8. *C. opacocorpa,* ventral view of anterior end, showing the arrangement of the cephalic gland ducts and spines on the dorsal lip of the oral sucker.
Fig. 9. *C. opacocorpa,* ventral view showing distribution of pigment.
Fig. 10. *C. opacocorpa,* lateral view of anterior end with oral sucker retracted.
Fig. 11. *C. opacocorpa,* lateral view of anterior end with oral sucker protruded.



Cercariae of Pleurolophocerca group.

STUDIES ON *DIENTAMOEBA FRAGILIS* (PROTOZOA). III. BINARY FISSION WITH SPECIAL REFERENCE TO NUCLEAR DIVISION

D. H. Wenrich
Zoological Laboratory, University of Pennsylvania

I. INTRODUCTION

In the two previous studies in this series on *Dientamoeba fragilis* (Wenrich, 1936, 1937a), special attention was given to the structure of the nucleus. The original observations of Jepps and Dobell (1918) and of other investigators were, in the main, confirmed, and additional facts and interpretations were also brought forward. In this third paper it is proposed to describe nuclear and cytoplasmic division and to discuss further the organization of the nucleus. A preliminary report was presented before the 1937 meeting of the American Society of Parasitologists (Wenrich, 1937b).

Although division stages had been seen occasionally while these studies were in progress, it was not until the summer of 1937 that more favorable material, consisting of a series of slides prepared by Dr. Robert M. Stabler, was examined. I am much indebted to Dr. Stabler for the privilege of studying these slides. Except for Figs. 28 to 37, all the drawings were made from this particular material. These smears had been fixed in modified Bouin's fluid (picro-formol-acetic: 75–15–10) and in Schaudinn's fluid plus either 5%, 10% or 20% of glacial acetic acid. There were no very pronounced differences among the slides prepared by the use of these different mixtures.

I am also indebted to Dr. A. D. Waltz for aid in securing material and to Dr. Sarah H. Stabler for technical assistance.

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II. OBSERVATIONS

A. *Normal Nuclear Divisions*

1. *Outline of nuclear division.* The nuclear division of *Dientamoeba fragilis* is mitotic so that it is appropriate to employ the usual terms—prophase, metaphase, anaphase and telophase—in describing it. Four chromosomes are formed and these divide longitudinally. As with dividing protozoan nuclei in general, initiation of prophase changes is accompanied by a swelling of the nucleus as a whole. An intranuclear division

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center elongates into a desmose which usually occupies the spindle axis during late prophases, metaphases, and anaphases. The nuclear membrane remains intact until the telophase constriction into two, after which the desmose still remains connecting the two daughter nuclei for some time.

The series of drawings on Plate I represent what is believed to be normal conditions. Those animals which are undergoing nuclear division (Figs. 3 to 23) are fairly uniform in size, varying from eight (Fig. 3) to eleven (Fig. 18) micra in diameter. An exception, an individual only five micra in diameter, is represented by Fig. 15.

2. *The interphase nucleus.* It has been difficult to identify definitely the "resting" or interphase condition of the nucleus. As previously described, the nucleus, as commonly seen, is from two to three micra in diameter and vesicular in structure. There is a central mass made up of several chromatic "granules" imbedded in a more faintly staining matrix. This is surrounded by a clear zone outside of which is a nuclear membrane, the latter having little or no chromatin on its inner surface. In the two previous studies the great range of variation in the appearance of the nuclei has been emphasized. While some of these variations probably represent certain stages in the normal cycle of nuclear changes, many of them are doubtless abnormal.

A logical approach to the interphase condition would be through the telophases. A late telophase stage might be expected in individuals undergoing plasmotomy (Figs. 24 to 27). The nuclei in Fig. 27 are more like those in the early telophase stages shown in Figs. 22 and 23, while those in Figs. 26, 25, and 24 apparently represent gradually more diffuse conditions in interphase nuclei. Fig. 1 is believed to represent a late telophase nucleus somewhat further along toward an interphase condition than the nuclei in Fig. 27. It will be noted that there are four chromatic areas in the central nuclear mass and that one of them, the "endosome," is more compact and more deeply stained than the others. In addition, Fig. 1 shows a small granule just inside the nuclear membrane attached to the "endosome" by a fine thread-like connection. This small granule is considered to be the division center and can be traced through the prophase stages until it elongates into an intranuclear desmose. Further resolution of the chromatic areas in the central mass is indicated in Fig. 2 where the component chromatic units of this mass are more or less fused together. This may be considered to be an interphase condition. A division center is also visible in this figure.

3. *Prophases.* Not very many spireme-like stages were found. Fig. 3 represents the nearest approach to such a stage. There is a more or less continuous chromatic thread, irregular as to thickness and density, disposed through various focal levels of the nucleus. The division center

is clearly visible. The spireme appears to condense gradually into a series of four chromatic masses which are the prophase chromosomes (Figs. 4 to 7). At first these are dumbbell in shape (Figs. 6 and 7), then as they elongate the median constriction changes to a longitudinal split. Eventually they assume a cross-like arrangement (Fig. 10) which appears to be a monopolar one under the influence of the division center (Fig. 9). As shown in Figs. 8, 9, and 10, one of the four chromosomes is shorter than the others. Many of the division stages show this shorter member, thus indicating persistent individual differences among the chromosomes.

In the late prophases the division center gradually elongates into a desmose as shown in Figs. 11 and 12; but the condition of the chromosomes in these figures suggests closer phase relations with the stages illustrated in Figs. 7 and 8. It is therefore difficult to give precise allocations in the series to a number of these stages. Eventually the desmose elongates until it seems to press against the nuclear membrane at one or both ends (Figs. 12, 13, and 14), after which the spindle axis seems to shorten as the metaphase stage is reached.

4. *Metaphase.* Stages interpreted as metaphases were fairly common on this set of slides. The shortened spindle axis of this stage suggests that the desmose may contract at this time (Figs. 15, 16, and 17). In some instances there is evidence that spindle fibers grow out from each individual chromosome (Fig. 16). In others there is a tendency for the whole group of chromosomes to contract into a fairly compact mass (Fig. 15). This latter condition could scarcely be considered normal, yet many examples were seen. As shown in Fig. 17, the chromosomes are oriented in the metaphase and early anaphase so as to bring about a longitudinal division of each of them. The irregular distribution of the chromosomes illustrated in Fig. 14 may indicate only a late orientation on the spindle or possibly an irregularity of orientation which might lead to unequal chromatin distribution to the two daughter nuclei. (See also Figs. 47 to 50.)

5. *Anaphase.* During anaphase the whole nucleus lengthens along the spindle axis, the desmose becoming longer and the daughter chromosomes segregating to opposite poles (Figs. 18 to 20). In late anaphase the desmose often takes a more peripheral position as shown in Fig. 20. Later anaphase stages (e.g., Fig. 20) were more common on the slides than the earlier ones (Figs. 18 and 19), suggesting a slowing of movement toward the end of nuclear elongation.

6. *Telophase.* During early telophase the nuclear membrane is constricted into two. The early telophase chromosomes are sometimes dense and clumped together making it difficult to count them (Fig. 22). The desmose persists for a considerable length of time after nuclear division,

since it is seen in many individuals in which the nuclei are in essentially an interphase condition.

7. Nuclear division from other sets of slides. Although the Stabler group of slides provided a fairly complete series of stages of divisions as shown on Plate I, comparable stages were found scattered through other sets, as illustrated in Figs. 28 to 37 (Plate II), each of which has been taken from a different series of slides. These figures reveal that division stages occur more or less sporadically even when there is no general tendency to division. However, as would be supposed, some groups of slides show more division stages than others and on some no divisions have yet been found.

B. *Cytoplasmic Division*

Plasmotomy, or division of the cell body, appears to take place by simple constriction. However, it apparently may be long delayed, in which case the desmose disappears and the cell remains binucleate for an unknown length of time. In other cases, plasmotomy takes place while the desmose is still persisting as shown in Figs. 24 to 27 and 37. The amount of nuclear change occurring before cell division seems to vary greatly. The nuclei in Fig. 27 appear to be more recently divided than those in Figs. 24 to 26.

C. *Unusual Nuclear Conditions*

Various unusual conditions of the nuclei have been found on the Stabler set of slides as well as on other sets. In the two previous papers, special attention was given to variations in nuclear conditions and many other series of slides have revealed similar conditions. Some of these unusual conditions are obviously abnormalities, while others can be variously interpreted. Figs. 38 to 54, drawn from the same slides as were Figs. 1 to 27, have been selected to show certain types of variations relating to reproduction.

Certain stages on these slides resembled some of those published by Brug (1936). Figs. 38 to 42 show a series of unusual anaphase stages similar to Brug's Figs. 4 to 8 which he had made from culture material. These drawings suggest that normal division processes may have been arrested at some stage followed possibly by some regressive changes.

Some of the difficulties in counting "granules" in the nuclei are illustrated by Fig. 43. The lower drawing, of the entire cell, shows an optical section of the nucleus and apparently nine "granules" can be counted. By focussing up and down, however, it can be seen that the various "granules" are really strands which are more or less connected with each other above and below, making a sort of spireme, as shown in the upper drawing of the nucleus alone. Thus it is often very difficult to determine the exact number of chromatic units in these nuclei.

Polyploidy is suggested by Figs. 44, 45, and 46. Fig. 44 shows a nucleus with apparently about a dozen "granules," seemingly an early prophase. A definite prophase with eight chromosomes is shown in Fig. 45. Fig. 46 depicts a telophase with nuclei which are larger than usual and which contain more than the usual number of granules. Interphase nuclei with eight or more "granules" are more common on some sets of slides than on others.

Unequal division of nuclei has previously been described (Wenrich, 1937a). Additional examples have been found on the Stabler set of slides. Fig. 47 represents a late prophase or a metaphase with an irregular distribution of chromosomes on the spindle. If such a division were completed it would result in nuclei of different sizes. Other examples of unequal divisions are illustrated in Figs. 48, 49, and 50.

It is to be noted that as a rule nuclear division takes place only in mononucleate individuals (Figs. 1–42). Many thousands of specimens were viewed before one was seen with more than two nuclei. Fig. 51 shows an individual with three nuclei, one about twice the size of either of the other two, which are connected by a desmose. A complete division of one nucleus and an incomplete division of the other are indicated. When segregated into a single cell, an incompletely divided nucleus as shown here might lead to polyploidy (cf. Fig. 45). In Fig. 52 a prophase condition (cf. Figs. 5 and 28) is seen in a binucleate individual. Figs. 53 and 54 show quadrinucleate specimens. In Fig. 53 one nucleus is degenerating. In Fig. 54 the four nuclei are linked in two pairs by desmoses. On another set of slides one individual with eight nuclei was observed.

III. DISCUSSION

A. *The Nuclear Division in* Dientamoeba

The foregoing account of nuclear division in this species reveals that it is definitely mitotic in character, yet the details appear to be distinctive. When compared to other intestinal amoebae the uniqueness of its division phenomena are as striking as the details of its nuclear organization. The small size of the nuclei (2 to 4 micra in diameter) renders the interpretation of finer details very difficult, but this difficulty is compensated for in part by the smallness of the chromosome number. It should be emphasized that most of the stages shown on Plate I were seen repeatedly so that the phenomena represented could be thoroughly corroborated. Further corroboration has been obtained from the occasional division stages on other sets of slides (e.g., Figs. 28 to 37).

Except for the abstract already published (Wenrich, 1937b), previous literature provides no similar account of division stages for *Dientamoeba.* Jepps and Dobell (1918) show in their Fig. 15 a condition

interpreted as a possible division stage. It is probably an abnormal one. Kudo (1926) in his Figs. 7 to 12 presented several stages of nuclear and cytoplasmic division but the chromosomal details were not made out. Brug (1936) described nuclear division in *Dientamoeba* from slides made from culture material. No real mitotic process is indicated in his illustrations although his Figs. 4 to 8 are somewhat like Figs. 38 to 42 of the present paper. Brug admits that his drawings could be interpreted as indicating amitosis, but suggests that his series may be incomplete. He saw no stages in plasmotomy. For the study of cytological details, intestinal protozoa obtained directly from the host are, I believe, preferable to those from cultures. Both Kofoid (1923) and Brug (1936) show specimens with four nuclei, but, as Brug points out, these are excessively rare. It would seem that the binucleate condition is the one which provides the best physiological balance for this species.

B. *The Interphase Nucleus and the Chromosomes*

As pointed out by Craig (1926) there is a marked tendency for the nuclei of *Dientamoeba* to show four "granules" in the condition commonly found on prepared slides. Since the present study reveals four chromosomes in mitosis, the question as to the relation between the interphase "granules" and the chromosomes immediately arises. When it is stated that four "granules" are present in the central nuclear mass of an interphase nucleus, it is to be understood that these "granules" are not always clearly defined homogeneous bodies. In many cases the chromatic units in these nuclei are in a partially diffused condition, although remaining blocked out into four chromatic areas. In telophase nuclei the four chromosomes remain compact and closely clumped together for some time (Figs. 21 and 22), and then begin to become more diffuse (Figs. 23 and 27). Further resolution makes it difficult to determine the exact number of chromatin units although there may still remain four centers of denser material (Figs. 1 and 25). In Fig. 2 where the whole inner nuclear mass appears to have formed a sort of central vesicle, four denser areas are still to be seen. Although it is difficult to recognize four units in the spireme stage (Fig. 3), in all the other prophase stages, the four units can usually be recognized. It is possible that the so-called matrix of the central nuclear mass represents the more diffuse portions of the interphase chromosomes and the "granules" the more compact portions.

A further question arises in connection with the tendency for more than four "granules" to appear in many nuclei. Actual division of the four primary units during interphase would result in more than four units. If these divisions were not simultaneous, numbers from five to eight would result, as noted in the first of these studies. As noted there, also, occasional fusion of nuclei—an obviously abnormal process—

would also result in eight or more units. Unequal divisions, such as shown in Figs. 30 to 32 of the second paper of this series and in Figs. 47 to 50 of the present paper, would naturally lead to more than four units in one of the daughter nuclei in each case. Incomplete nuclear division, as indicated in Fig. 51, or a complete division of chromatin units between mitosis might result in polyploidy. Figs. 44, 45, and 46 suggest this condition.

Multiplicity of the "granules" in interphase nuclei does not necessarily indicate increases in fundamental chromatin units. In the partial resolution process of the late telophases, each former chromosome could be represented by two or more condensed regions, since the resolution seems normally to be incomplete. On the other hand a telophase separation into two components might occur in anticipation of the next division, to be followed by reassociation in the succeeding prophase. With all these possibilities in mind it is difficult to interpret many of the conditions actually found.

C. *The Division Center*

The division center in the nuclei of *Dientamoeba* is a very small body and was overlooked until the origin of the desmose was sought. In the early prophases it is sometimes apparently against the nuclear membrane and sometimes attached to one of the prophase chromosomes by a very fine connecting strand.

The problem of the continuity of the division center is a puzzling one. In Fig. 18, showing an anaphase, the desmose has definite granules at each end. It has not been possible to follow the history of these granules through the later anaphases and telophases. It was not possible to determine, therefore, whether these daughter division centers remain as independent granules when the desmose fades away, or become merged with the "endosome" or disappear. Conditions illustrated in Figs. 23, 37, 46, 53, and 54 suggest the persistence of a centriole-like granule apparently imbedded in or attached to the "endosome." If the interpretations of Fig. 1 as a telophase and of Fig. 2 as an interphase are correct, then the persistence of a division center is indicated. Numerous specimens containing two nuclei connected by a persisting desmose were examined to see whether the desmose retained a connection with the endosome or with a granule on the nuclear membrane. The results were inconclusive, since in many cases the desmose was too faint in the vicinity of the nuclei to be traceable (Figs. 26, 27, and 50). In other cases connection with the endosome seemed to persist (Fig. 23), while in still others a small granule on the nuclear membrane was seen (Figs. 25 and 54), which sometimes appeared to be connected with the endosome (Fig. 54). In Figs. 1, 4, 5, and 6, similar connections between a periph-

eral granule and the endosome are shown, but in Figs. 2 and 3 a connection could not be made out. While these apparently persisting "centrioles" were not seen with sufficient uniformity to justify an unqualified statement as to their persistence, yet the fact that they could be found in a good many cases suggests that there is a continuity of identity for this structure from one division to the next.

D. *The "Endosome"*

In the first and second papers of this series emphasis was placed on the fact that one of the "granules" of the central nuclear mass of *Dientamoeba* often behaves differently from the others, frequently being larger, or more deeply stained, or both. There was, however, great variation in size in relation to the whole nucleus and in relation to the rest of the central mass. Since this "endosome" is one of the four "granules" that commonly appear in the central nuclear mass and since four chromosomes appear in mitosis, it seems probable that the "endosome" may also represent a chromosome. The chromosomal nature of the "granules" in the central nuclear area was not suspected at the time the two previous papers were written. Now that the "endosome" appears to constitute also a chromosome, its endosomal status becomes questionable. However, its differential behavior calls for an explanation. If it is also the locus of the division center or at least closely linked to the division center, its differential behavior becomes more understandable. Its variations in comparative size might be related to a dual composition. In those nuclei where it is relatively small (See Wenrich, 1937a, Figs. 2, 4, 5, 6, 18, 20, and 21) possibly the chromosomal component has become diffuse while the centriolar part remains compact; in those cases where it is relatively large (Wenrich, 1937a, Figs. 7, 8, 9, 10, 11, 12, 13, 14, 23, and 24) more or less of the chromosomal constituent may remain condensed along with the centriolar component. On the other hand, in the nuclei of many individuals there is little in the way of differential behavior on the part of the "endosome" (Figs. 24 and 25), and this is also true during the process of mitosis, where it has not been possible to determine which of the four chromosomes includes or is derived from the interphase "endosome." Further study may shed light on this problem.

IV. SUMMARY

1. The nuclear division of *Dientamoeba fragilis* is mitotic, involving the formation of four chromosomes and the development of a desmose from an intranuclear division center.

2. Nuclear division is limited primarily to mononucleate individuals of fairly uniform medium size. A very few larger individuals with four or more nuclei have been found.

3. Prophases begin with a rough spireme which condenses into four dumbbell-shaped chromosomes. These gradually elongate and may show a longitudinal split. They then assume a monopolar arrangement oriented toward the division center. As the division center lengthens into a desmose the prophase chromosomes become arranged in the metaphase plate. One of the chromosomes is smaller than the others, thus illustrating chromosomal individuality.

4. In metaphase the spindle axis seems to shorten somewhat and the chromosomes often appear to be clumped on the spindle. Other examples show an orientation which ensures a longitudinal division of the chromosomes. In some cases each chromosome appears to send out its own spindle fibers.

5. As the nucleus elongates during the anaphase the desmose also lengthens and the daughter chromosomes migrate to opposites poles. In later anaphases the desmose often assumes a more peripheral position.

6. In early telophase the nuclear membrane constricts into two, but the desmose persists for some time connecting the two daughter nuclei. Telophase chromosomes at first are compact and crowded together, but they gradually expand and become more diffuse as they pass into the interphase stage.

7. The interphase nucleus usually shows four or more condensed chromatin areas imbedded in a less chromatic matrix. It is suggested that the matrix consists of the more diffuse portions of the interphase chromosomes.

8. More than four "granules" in the interphase nucleus may be accounted for in a variety of ways: (a) two or more denser areas may occur in the same interphase chromosome; (b) telophase splitting may occur in anticipation of the succeeding mitosis; (c) incomplete nuclear division (Fig. 51), or occasional nuclear fusion, may lead to polyploidy; (d) unequal division may produce some nuclei with extra chromosomes.

9. Plasmotomy may take place soon after nuclear division and before the desmose has disappeared or it may be deferred, leading to binuclearity. Because of its numerical predominance, the binucleate condition is probably more stable physiologically than the mononucleate.

10. Although the details of mitosis were first worked out on one series of slides, many division stages have been found on other sets.

11. Some unusual mitotic figures resemble those drawn by Brug (1936) from culture material. There is doubt as to their being normal.

12. The evidence in this study indicates the persistence of the division center from one mitosis to the next. This division center seems to be connected with the "granule" which was previously interpreted as an "endosome." The differential behavior of the "endosome" may thus be accounted for, although it also appears to develop into one of the chromosomes during mitosis.

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VI. EXPLANATION OF PLATES

All figures have been drawn with the aid of a camera lucida at an initial magnification of ×3000 and reduced about ⅛ in printing. Figs. 1 to 27 and 38 to 54 were all made from the same set of slides. Figs. 28 to 37 were each made from a different set of slides. Figs. 5, 16, 31, 32, 33, 34, 36, 37, 38, and 54 were made from slides fixed in Bouin's fluid; Fig. 35 from a slide fixed in Hollande's fluid; all others from slides fixed in Schaudinn's fluid with 5% to 20% of glacial acetic acid added.

Plate I

Figs. 1 to 27 represent what is thought to be a normal series of stages in nuclear and cell division of *Dientamoeba fragilis*.

Fig. 1. Mononucleate individual, late telophase stage in nucleus; four chromatic areas; connection between division center and "endosome."

Fig. 2. Nucleus in interphase; division center visible.

Fig. 3. Early prophase spireme; division center visible.

Fig. 4. Early prophase; early condensation of spireme; division center on nuclear membrane.

Fig. 5. Early prophase; further condensation of spireme; division center on nuclear membrane.

Fig. 6. Early prophase; four chromosomes showing; one attached to division center.

Fig. 7. Later prophase; each chromosome shows a constriction; division center seen.

Fig. 8. Later prophase; chromosomes longer, show longitudinal split.

Fig. 9. Later prophase; monopolar orientation of chromosomes toward division center.

Fig. 10. Later prophase; chromosomes in "cross" arrangement; division center beginning to elongate.

Fig. 11. Later prophase; chromosomes more clumped; division center elongating.

Fig. 12. Later prophase; desmose well-developed; chromosomes becoming oriented for metaphase.

Fig. 13. Early metaphase; spindle slightly asymmetrical.

Fig. 14. Early metaphase, belated orientation (?); or early anaphase, unequal division (?).

FIG. 15. Metaphase; small cell, 5 μ in diam.; chromosomes clumped; spindle axis short.
FIG. 16. Metaphase; spindle fibers apparently growing out from each chromosome.
FIG. 17. Early anaphase; longitudinal split in chromosomes indicated.
FIG. 18. Anaphase; division center granules at each end of desmose.
FIG. 19. Anaphase; division center granules not seen.
FIG. 20. Late anaphase; desmose has become peripheral.
FIG. 21. Early telophase; chromosomes crowded together; desmose persisting.
FIG. 22. Telophase; chromosomes clumped; desmose persists.
FIG. 23. Telophase; nuclei beginning to expand, chromosomes beginning to undergo resolution.
FIGS. 24 to 27. Stages in plasmotomy; desmose persists. Gradual resolution of chromosomes to interphase condition, Figs. 27 to 26 to 25 to 24.

PLATE II

FIGS. 28 to 37. A series of division stages, each from a different set of slides.
FIG. 28. Early prophase; condensation of "spireme" (cf. Fig. 4).
FIG. 29. Later prophase; individual chromosomes, each with a constriction (cf. Fig. 7).
FIG. 30. Later prophase; chromosomes condensed; some indication of longitudinal split (cf. Fig. 8).
FIG. 31. Late prophase; "cross" arrangement of chromosomes (cf. Fig. 10).
FIG. 32. Late prophase; monopolar arrangement of chromosomes (cf. Fig. 9).
FIG. 33. Metaphase; spindle axis shortened, chromosomes clumped (cf. Fig. 15).
FIG. 34. Early anaphase (cf. Fig. 18).
FIG. 35. Later anaphase; desmose peripheral (cf. Fig. 20).
FIG. 36. Early telophase; daughter nuclei rounding up; desmose persists.
FIG. 37. Early stage of plasmotomy (cf. Fig. 26).
FIGS. 38 to 42. Unusual and probably abnormal anaphases similar to Brug's (1936) Figs. 4 to 8; same slides as Figs. 1 to 27.
FIG. 43. Two views of same nucleus at different focal levels; lower drawing shows optical section, apparently 9 "granules"; upper drawing shows connection of "granules" at upper and lower focuses.
FIGS. 44 to 46. Evidence for polyploidy.
FIG. 44. Early prophase (?); more "granules" than usual.
FIG. 45. Definite prophase with 8 chromosomes.
FIG. 46. Telophase; large nuclei with more than usual number of "granules."
FIG. 47. Asymmetrical metaphase; possible prelude to unequal nuclear division.
FIGS. 48 to 50. Telophases with nuclei of unequal size.
FIG. 51. Trinucleate individual; incomplete division of larger nucleus; possible prelude to polyploidy.
FIG. 52. Prophase in binucleate individual (cf. Fig. 5).
FIG. 53. Individual with 4 nuclei, one degenerating.
FIG. 54. Individual with 4 nuclei, connected in pairs by desmoses.

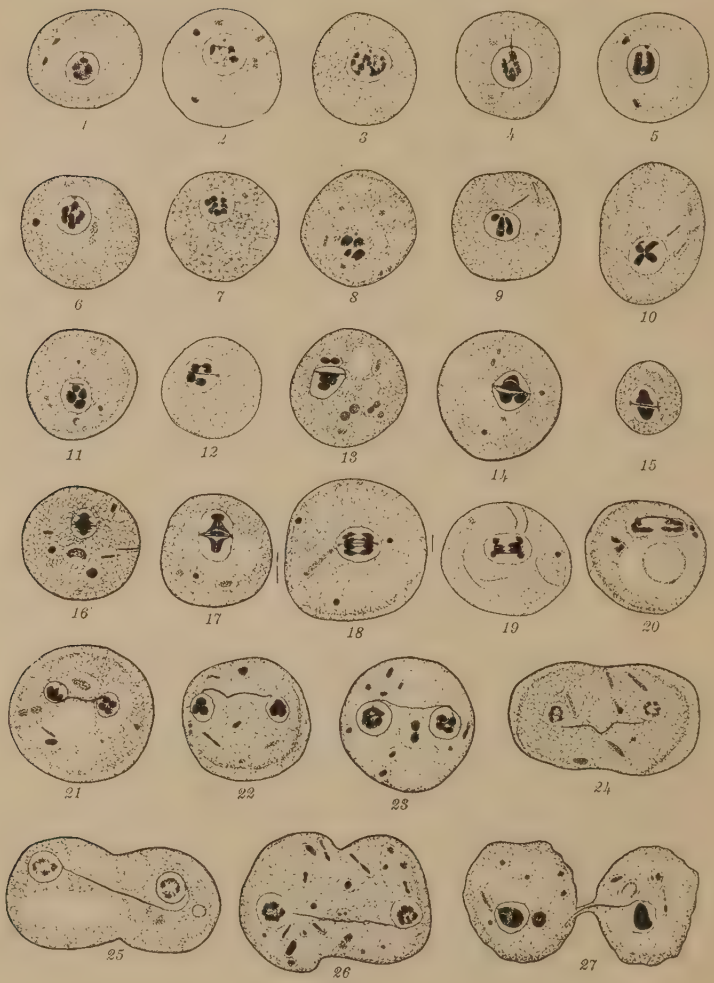

PLATE I

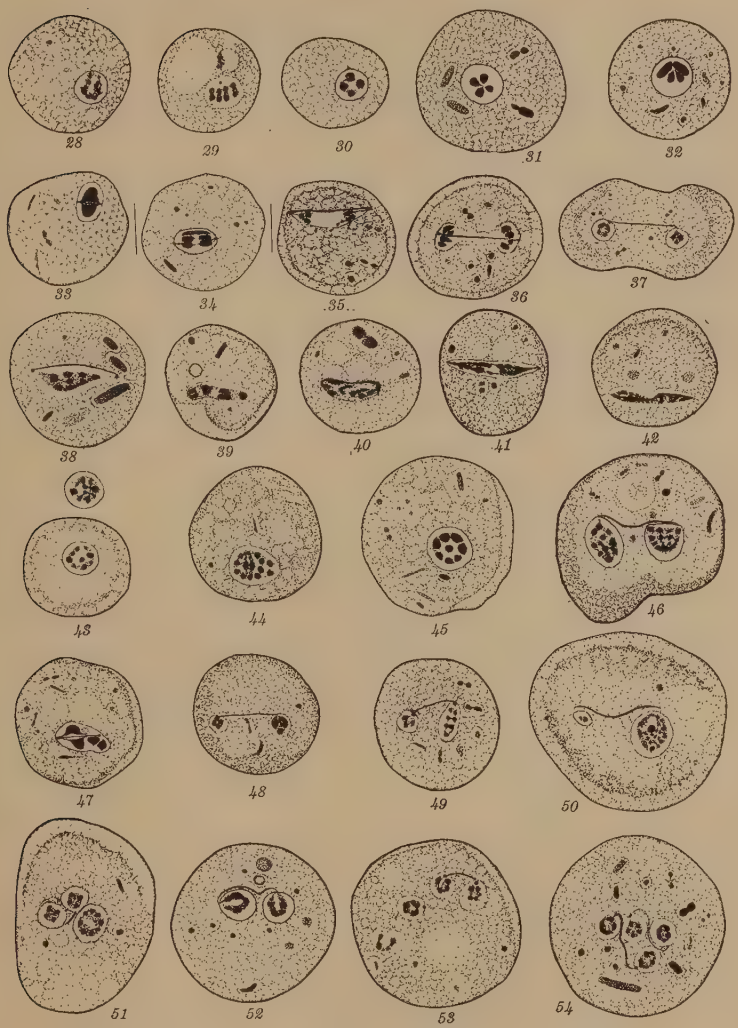
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PLATE II

ACQUIRED IMMUNITY TO TICKS

WILLIAM TRAGER
Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.

It is well known that the American dog tick, *Dermacentor variabilis* Say, can be reared through its entire life cycle on guinea pigs. In the course of permitting successive batches of larvae of this tick to engorge on the same guinea pig, it was repeatedly observed that, while the first batch always gave a large number of engorged larvae, the following batches gave few or none. This suggested that guinea pigs, after one infestation with larvae of *D. variabilis,* acquire an effective immunity against these ectoparasites. The experiments reported in this paper show that such an immunity is indeed developed, and that it may be solid enough to prevent larvae from engorging and to reduce the amount of blood taken by nymphs and adults.

METHODS

Guinea pigs were used for most of the experiments, rabbits and deer mice (*Peromyscus leucopus*) for a few. In order to permit repeated observation and careful control of the engorging ticks the pill box method (Kohls, 1937) has been employed in all the experiments except those concerned with deer mice. One and one-half or one and one-quarter-inch pill boxes with the bottoms cut out were attached to the previously clipped sides and over the ears of guinea pigs by means of adhesive tape (Plate I, Fig. 1). Rabbits were treated in the same manner except that the ears were fitted with tall cylindrical boxes with a hole cut in the bottom just large enough for the ear to pass through. Counted numbers of ticks were placed in the boxes and observed at intervals during the course of engorgement. Engorged ticks which had dropped off could be readily recovered from the boxes and counted. Occasionally, an animal would succeed in scratching off its ear box. In order to prevent the escape of ticks freed in this manner, all animals with ticks on them were kept in cages made out of one-half-inch mesh wire screen and completely surrounded by a cloth bag tied in front (Kohls, 1937).

The deer mice were trapped in woods near Plainsboro, N. J., during the latter part of March and hence before they could have been infested by larvae or nymphs of *D. variabilis.* They were kept individually in glass battery jars with shavings, and were fed oats, Purina dog chow and water. In order to infest a mouse, a counted number of larvae was sprinkled over it while it was held in a large white pan. All the ticks

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which fell into the pan were gathered up on a piece of cotton which was put in the jar together with the mouse. The mouse would nest in the cotton, increasing its chances of infestation. Each jar was provided with vaseline at the rim and a wire screen cover, and was held in a large white pan containing water, which caught the few ticks able to get past the vaseline. Two days after the application of the ticks, each mouse was transferred to a fresh jar. Hot water was run into the used jar. Two days still later, each mouse was again placed in a fresh jar. This time, the contents of the old jar were carefully examined for engorged larvae. This process was repeated every other day until all the larvae had dropped from the mouse (usually the eighth day after their application).

The general plan of the experiments with guinea pigs and rabbits has been to use litter mates, usually of the same sex and color, to immunize one and then, after a suitable interval, to apply to both animals ticks descended from the same female and kept under identical conditions. In some experiments litter mates were not used, but a suitable untreated control was always infested at the same time as the animal being tested for immunity, and with ticks from the same batch. Similarly, in each series of infestations of deer mice, larvae from the same batch were used. It is believed that in this manner the great variability in infesting power of different batches of ticks and of the same batch at different times was sufficiently controlled.

RESULTS

I. *Immunity to Larvae.* Table 1 shows that after one infestation of either a guinea pig or rabbit with larvae or nymphs of *D. variabilis* the number of larvae which can subsequently engorge on the animal is greatly reduced. Exper. 15b indicates that the immunity is not a strictly local one. Guinea pig (hereafter abbreviated in text and tables as GP) 29, which had had two infestations with nymphs applied only on the sides of its body, completely resisted the attack of larvae placed on its ear. Tested similarly, GP 41 was partially immune: after one infestation with larvae on its sides only started 7 days previously, 11 out of 100 larvae engorged, but were smaller than normal.

Exper. 16 gives further data on the development and spread of the immunity. The engorgement of only 20 larvae on the left ear of GP 31 rendered both ears partially immune one week later, and both ears and the left side completely immune 12 days after the start of the first infestation. GP 32, litter mate of GP 31, which wore only a box on its left ear during the first infestation of GP 31, was highly susceptible, 51 out of 100 larvae engorging in a normal manner. Exper. 17 shows again the spread of the immunity from the left to the right ear. The first part of Exper. 22 shows that one infestation on the sides of the body rendered

TABLE 1.—*Acquired immunity to larvae of* Dermacentor variabilis

Exper. No.	Animal	Previous treatment	Region of body*	Ticks put on		No. engorged	Remarks†
				No.	Date		
26	GP 46 ♀	None	L.S.	100	3/ 9/38	18	
			R.S.	100	"	14	
			L.E.	100	"	64	
			R.E.	100	"	77	
		1 infestation started on 3/9/38	L.E.	100	3/15/38	1	Small and brownish
	GP 47 ♀ Litter mate of 46	Wore boxes only, for same period as 46	L.E.	100	"	33	
28	Rabbit 1 ♂	None	L.S.	100	3/11/38	6	
			R.S.	100	"	29	
		1 infestation started on 3/11/38	L.S.	114	3/25/38	0	
			R.S.	86	"	0	
	Rabbit 2 ♂ Litter mate of 1	Wore boxes only, from 3/11 to 3/18/38	L.S.	86	"	10	
			R.S.	114	"	12	
15	GP 29 ♀	1 infestation with nymphs, 1/13 to 1/23/38	L.S.	50	2/ 1/38	0	
	GP 30 ♀ Litter mate of 29	Wore boxes only from 1/13 to 1/23/38	L.S.	50	"	8	
15a	GP 29 ♀	2 infestations with nymphs on sides of body, last on 2/1 to 2/11/38	L.S.	100	2/24/38	0	
			R.S.	100	"	1	Light brown
	GP 41 ♀	None	L.S.	100	"	29	
			R.S.	100	"	34	
15b	GP 29 ♀	As in 15a. Immune to larvae on sides	L.E.	100	3/ 3/38	0	
	GP 41 ♀	1 infestation with larvae just ending	L.E.	100	"	11	Smaller than normal
16	GP 31 ♀	None	L.E.	100	1/13/38	20	
		1 infestation on left ear, 1/13 to 1/18/38	L.E.	100	1/20/38	4	All 7 engorged larvae of a light brown color
			R.E.	100	"	3	
	GP 32 ♀ Litter mate of 31	Box only, on left ear, 1/13 to 1/18/38	L.E.	100	"	51	
	GP 31	1 infestation on left ear, almost immune on both ears on 1/20	L.S.	50	1/25/38	0	
			L.E.	50	"	0	Dead attached unfed larvae noted on both ears
			R.E.	50	"	0	
	GP 32	1 infestation on left ear, box on right ear 1/20 to 1/24/38	L.S.	50	"	0	
			L.E.	50	"	2	One of the 3 engorged larvae was light red, the other 2 undersized and dark brown
			R.E.	50	"	1	
17	GP 33 ♂	None	L.E.	100	1/18/38	15	
		1 infestation on left ear 1/18 to 1/25/38	L.E.	100	1/25/38	0	Several attached but dead larvae noted
			R.E.	100	"	10	
	GP 34 ♂ Litter mate of 33	Box only on left ear 1/18 to 1/25/38	L.E.	100	"	30	

* L.S., left side; R.S., right side; L.E., left ear; R.E., right ear.
† Remarks omitted when the engorged larvae were of normal size and color.

TABLE 1.—*Continued*

Exper. No.	Animal	Previous treatment	Region of body*	Ticks put on		No. engorged	Remarks†
				No.	Date		
22	GP 12 ♂	1 infestation around center of body on 11/16 to 11/23/37. Immune on 12/1/37	L.S.	100	2/14/38	0	
			L.E.	100	"	2	Both engorged larvae brown
	GP 39 ♀	None	L.S.	100	"	13	
			L.E.	100	"	26	
		1 4-day-old infestation	R.S.	100	2/18/38	25	
			R.E.	100	"	30	
	GP 40 ♀ Litter mate of 39	Boxes on sides and left ear starting on 2/14/38	R.S.	100	"	49	
			L.E.	100	"	58	
		1 infestation started on 2/18/38	L.S.	100	2/23/38	0	
			R.E.	100	"	35	A few of these were dark brown rather than black
35	GP 46 ♀	1 infestation with larvae on both sides and both ears. 1 infestation with nymphs 3/15 to 3/25/38	L.S.	100	4/ 6/38	0	
			L.E.	100	"	3	7 originally seen attached. Of the 3 engorged, 1 was reddish brown
	GP 47 ♀ Litter mate of 46	1 infestation with nymphs and larvae on sides and ears 3/15 to 3/25/38	L.S.	100	"	0	
			L.E.	100	"	17	3 normal, 6 undersized, 6 undersized and brown, 2 very pale
	GP 30 ♀	1 infestation with larvae and nymphs on left side only 2/1 to 2/11/38	L.S.	100	"	0	
			L.E.	100	"	41	Several of these very pale
	GP 60 ♀	None	L.S.	100	"	12	
			L.E.	100	"	58	
35a	GP 46 ♀	1 infestation with larvae. 2 with nymphs. Last on 4/6 to 4/16/38	L.E.	100	4/26/38	3	1 of these undersized
	GP 47 ♀	1 infestation with nymphs and larvae. 1 with nymphs on 4/6 to 4/16/38	L.E.	100	"	2	8 originally seen attached. Of the 2 engorged, 1 was brown, other undersized
	GP 30 ♀	2 infestations with larvae and nymphs, last on 4/6 to 4/16/38, when partly immune to larvae	R.E.	100	"	11	4 undersized, 1 light red, 6 very pale. Large blister at site of attachment of a cluster of ticks most of which died when only slightly engorged
	GP 62 ♀	None	R.E.	100	"	72	
36	GP 32 ♀	1 infestation on left ear only. Immune on 1/25/38	R.S.	100	4/21/38	2	
			L.E.	100	"	36	A few were brown or undersized
	GP 61 ♀	None	R.S.	100	"	62	
			L.E.	100	"	99	
42	GP 36 ♀	1 small infestation with larvae on left side only, 2/1 to 2/7/38	L.S.	100	5/19/38	0	
			L.E.	100	"	5	Undersized. Some others attached but died without feeding
			R.E.	100	"	5	

* L.S., left side; R.S., right side; L.E., left ear; R.E., right ear.
† Remarks omitted when the engorged larvae were of normal size and color.

TABLE 1.—*Continued*

Exper. No.	Animal	Previous treatment	Region of body*	Ticks put on		No. engorged	Remarks†
				No.	Date		
	GP 50 ♀	Nymphs on right side, ear, larvae on left side, ear from 3/25 to 4/4/38	L.S.	100	"	0	
			L.E.	100	"	0	Few attached on both ears but failed to feed
			R.E.	100	"	0	
	GP 55 ♂	Nymphs on both sides, larvae on left ear, 4/16 to 4/26/38	L.S.	100	"	0	
			L.E.	100	"	0	About 16 attached but dropped off without engorging
			R.E.	100	"	3	Very small. Small blisters on ear
	GP 61 ♀	1 infestation with larvae on right side and left ear 4/21 to 4/27/38	L.S.	100	"	0	
			L.E.	100	"	1	Several others attached but dropped off without feeding
			R.E.	100	"	5	Undersized. Blisters on both ears
	GP 68 ♀	None	L.S.	100	"	28	
			L.E.	100	"	65	
			R.E.	100	"	57	

* L.S., left side; R.S., right side; L.E., left ear; R.E., right ear.
† Remarks omitted when the engorged larvae were of normal size and color.

the ear as well as the sides immune. In the case of GP 39, the right side and right ear, 4 days after the start of infestations on the left side and left ear, already showed some immunity as compared with the litter mate GP 40.

The result with GP 12 in Exper. 22 indicates that the immunity lasts at least 3 months. This is borne out by the data in Exper. 35, 36, and 42. These experiments also show clearly that the immunity is more effective on the sides of the body than on the ears. Even on previously untreated animals, however, usually a higher percentage of larvae was able to engorge on the ears than on the sides. GP 30 (Exper. 35), infested on its left side early in February, was still completely immune on the left side 2 months later, but only partially immune on its left ear. Forty-one larvae, some of them very pale in color, engorged on the ear of GP 30 as compared with 58 normal black larvae on the ear of control GP 60. However, when larvae were applied to the right ear of GP 30 (Exper. 35a) 10 days after the end of a fourth infestation with nymphs, running concurrently with the larval infestation on its left ear, only 11 engorged, none of them normal, as compared with 72 normal engorged larvae on the right ear of control GP 62.

In Exper. 36, the data on GP 32 show that this animal retained a partial immunity for 3 months. GP 36 (Exper. 42), which had one small infestation early in February on its left side only, showed, in the middle of May, almost as much immunity on its sides and ears as GP

50, 55 and 61, last infested in April. The results of Exper. 42 are perhaps especially striking in that they show the very great difference in number of engorged larvae obtained from a previously uninfested animal (GP 68) and from four different animals having one thing in common, namely, that they had been previously infested at least once with larvae or nymphs of *D. variabilis*.

A few experiments with larvae of *D. variabilis, Haemaphysalis leporis palustris* Packard[1] and *Dermacentor andersoni* Stiles[2] have shown that each of these species induces some immunity to itself and to larvae of the other species. Table 2 shows the cross immunity between *D. vari-*

TABLE 2.—*Cross-immunization between larvae of* Dermacentor variabilis *and* Haemaphysalis leporis palustris

Exper. No.	Animal	Previous treatment	Region of body*	No. larvae put on of		Date put on	No. engorged
				H. leporis palustris	*D. variabilis*		
39	Rabbit 3	None	L. S.	0	300	5/ 9/38	16
		1 infestation just ending	R. E.	0	200	5/17/38	44
		2 successive infestations with *D. variabilis*	L. S.	100	0	5/26/38	1
			R. S.	0	100	"	0
			L. E.	0	100	"	3
			R. E.	100	0	"	1
	Rabbit 4 Litter mate of 3	Boxes only, at same time as Rabbit 3	L. S.	100	0	"	8
			R. S.	0	100	"	1
			L. E.	0	100	"	72
			R. E.	100	0	"	78
	Rabbit 5	None	L. S.	300	0	5/ 9/38	42
		1 infestation just ending	R. E.	200	0	5/17/38	12†
		2 successive infestations with *H. leporis palustris*	L. S.	0	100	5/25/38	13
			R. S.	100	0	"	5
	Rabbit 6 Litter mate of 5	Boxes only, at same time as Rabbit 5	L. S.	0	100	"	26
			R. S.	100	0	"	23

* L. S., left side; R. S., right side; L. E., left ear; R. E., right ear.
† Got ear box off on first day.

abilis and *H. leporis palustris*. Two litter mate pairs of rabbits were used. One rabbit of one pair was infested with *D. variabilis*, one rabbit of the other pair with *H. leporis palustris*. Subsequently, both species of ticks were placed on both rabbits of each pair. In each case, the previously infested rabbit showed a high degree of immunity to both species.

Table 3 shows the cross immunity between *D. variabilis* and *D. andersoni*. Of three litter mate guinea pigs, one was infested with *D. variabilis* and a second with *D. andersoni*. All three were then infested with both species and again the previously infested animals showed partial immunity to both. The animal infested with *D. variabilis* on its left side and left ear subsequently gave 11, 11 and 19 engorged *D. andersoni*

[1] The larvae of *H. leporis palustris* were descended from a single engorged female obtained from an Oklahoma cottontail rabbit received by Dr. R. E. Shope.

[2] The larvae of *D. andersoni* were descended from females which were sent me in the unfed state through the kindness of Dr. R. R. Parker.

TABLE 3.—*Cross-immunization between larvae of* Dermacentor variabilis *and* Dermacentor andersoni

Exper. No.	Animal	Previous treatment	Region of body*	No. larvae put on of		Date put on	No. engorged
				D. andersoni	*D. variabilis*		
46	GP 77 ♀	None	L. S.	0	100	6/ 8/38	31
			L. E.	0	100	"	38
	GP 78 ♂	None	R. S.	100	0	"	10
	Litter mate of 77		R. E.	100	0	"	3
	GP 77	1 infestation	L. S.	100	0	6/21/38	11
	"	with *D. variabilis* from	R. S.	0	100	"	3
			L. E.	100	0	"	11
		6/8 to 6/15/38	R. E.	100	0	"	19
	GP 78	1 light infestation with *D. andersoni* from 6/8 to 6/15/38	L. S.	100	0	"	19
			R. S.	0	100	"	1
			L. E.	0	100	"	60
			R. E.	0	100	"	2
	GP 79 ♂	Wore boxes	L. S.	100	0	"	64
	Litter mate	only, from	R. S.	0	100	"	55
	of 77, 78	6/8 to	L. E.	100	0	"	22
		6/15/38	R. E.	0	100	"	81

* L. S., left side; R. S., right side; L. E., left ear; R. E., right ear.

on its left side, left ear and right ear respectively, as compared with 64 and 22 on the left side and left ear of the control. GP 78 underwent on its right side and right ear only a very light original infestation with *D. andersoni* (the larvae used for this infestation, unlike those used for the test infestations, were from a weak batch found to have difficulty in engorging even on previously uninfested animals). Nevertheless, the test infestation gave only 1, 60 and 2 engorged *D. variabilis* on the animal's right side, left ear and right ear respectively, as compared with 55 and 81 on the right side and right ear of the control. In GP 78 there was only slight spread of the immunity to the previously uninfested left ear.

Neither guinea pigs nor rabbits are natural hosts of larvae of *D. variabilis*. Hence it became important to discover whether a natural host, such as the deer mouse (Parker, Philip, Davis and Cooley, 1937), could develop an immunity to the tick larvae. A number of experiments showed that one infestation with larvae of *D. variabilis* induced no immunity and indeed even appeared to make the animal more susceptible. Two or three infestations did however induce an effective immunity, as shown in table 4. In two separate tests, mice 2 and 3, which had had three and two, and four and three, infestations respectively, supported the engorgement of fewer larvae than did the control mice. It is likely that deer mice never become completely immune. However, the relatively immune animals showed a noticeable difference in appearance from the susceptible animals. Plate I, Fig. 2, is a photograph of deer mouse 2 on the 5th day of its fourth infestation. Plate I, Fig. 3, shows deer mouse 6 on the 5th day of its first infestation. This mouse, before ticks had been applied to it, had a coat of fur even more sleek and smooth than

that of mouse 2. Mouse 2, during its first three infestations, and all mice during their first infestations, presented the same appearance as mouse 6. The fur became very wet and ruffled, probably from the excessive washing given it by the mouse in its attempts to dislodge the ticks. Several days after the larvae had all dropped off, the fur again became normal. In the series of infestations started on June 14, 1938 (Table 4), mouse 2, undergoing its fifth infestation, retained sleek smooth fur.

TABLE 4.—*The development by deer mice of acquired immunity to larvae of* Dermacentor variabilis

Deer mouse No.	Previous treatment	Ticks put on		No. engorged
		No.	Date	
2	None	300	4/13/38	29
2	1 infestation: 4/13 to 4/20	400	4/27/38	80
3	None	400	"	33
2	2 infestations: 4/13 to 4/20 and 4/27 to 5/4	400	5/14/38	33
3	1 infestation: 4/27 to 5/4	400	"	54
5	None	400	"	38
2	3 infestations: 4/13 to 4/20, 4/27 to 5/4, and 5/14 to 5/21	400	5/31/38	10
3	2 infestations: 4/27 to 5/4, and 5/14 to 5/21	400	"	8
6	None	400	"	69
2	4 infestations: 4/13 to 4/20, 4/27 to 5/4, 5/14 to 5/21, and 5/31 to 6/7	400	6/14/38	12
3	3 infestations: 4/27 to 5/4, 5/14 to 5/21, and 5/31 to 6/7	400	"	22
5	1 infestation: 5/14 to 5/21	400	"	37
7	None	400	"	185
11	None	400	"	118

The fur of mouse 3 was rather wet and ruffled, while that of mice 5, 7, and 11 was decidedly so. The fact that mice 3 and 5 showed wet ruffled fur in spite of the much fewer numbers of larvae which engorged on them than on mice 7 and 11 suggests that on the former partially immune animals many more larvae attached and started to engorge than were able to complete engorgement.

II. *Immunity to Nymphs and Adults of* D. variabilis. In these experiments, the nymphs placed on corresponding regions of test and control animals were always descendants of the same female, and, as larvae, had engorged on the same susceptible guinea pig at the same time. Early in the course of the work it was observed that previous infestations with larvae or nymphs had little or no effect on the number of nymphs able to attach and engorge. There seemed to be some effect on the size, however. A series of experiments, some of which are presented in table 5, has given entirely consistent results and shown that the engorged nymphs dropping from previously infested guinea pigs weigh less than the corresponding nymphs from control guinea pigs. The average weight of nymphs engorged on control guinea pigs was always about 10 mg. As may be seen in table 5, there was a fair correlation between the

TABLE 5.—*Acquired immunity to nymphs of* Dermacentor variabilis

Exper. No.	Animal	Previous treatment	Region of body*	Ticks put on		Engorged ticks	
				No.	Date	No.	Av. wt.
							mg
15	GP 29 ♀	2 infestations with nymphs starting on 1/13 and 2/1/38	L.S.	15	2/24/38	13	3.9
			R.S.	16	"	15	3.7
	GP 41 ♀	None	L.S.	15	"	14	10.1
			R.S.	16	"	15	9.7
32	GP 16 ♂	2 successive infestations with larvae. Immune to larvae on 12/24/37	R.S.	20	3/25/38	18	9.3
	GP 24 ♂	3 infestations with nymphs started on 12/14/37, 1/3 and 1/17/38	R.S.	20	"	20	6.4
	GP 29 ♀	3 infestations with nymphs started on 1/13, 2/1 and 2/24/38	R.S.	20	"	16	6.5
	GP 50 ♀	None	R.S.	20	"	18	12.5
32a	GP 16 ♂	Immune to larvae on 12/24/37. 1 infestation with nymphs started on 3/25/38	L.S.	20	4/16/38	15	6.7
			R.S.	20	"	19	7.5
	GP 24 ♂	4 infestations with nymphs started on 12/14/37, 1/3, 1/17 and 3/25/38	L.S.	20	"	15	4.7
			R.S.	20	"	19	5.8
	GP 29 ♀	4 infestations with nymphs started on 1/13, 2/1, 2/24 and 3/25/38	L.S.	20	"	16	3.5
			R.S.	20	"	2	1.7
	GP 55 ♂	None	L.S.	20	"	18	12.7
			R.S.	20	"	19	10.7
35	GP 46 ♀	1 infestation with larvae, 2 with nymphs started on 3/15 and 4/6/38	L.S.	20	4/26/38	14	5.4
			R.S.	15	"	14	4.4
	GP 47 ♀ Littermate of 46	1 infestation with nymphs and larvae, 1 with nymphs, started on 3/15 and 4/6/38 respectively	L.S.	20	"	13	6.2
			R.S.	15	"	11	7.2
	GP 30 ♀	1 infestation with nymphs and larvae, 1 with nymphs, started on 2/1 and 4/6/38 respectively	L.S.	20	"	16	2.6
			R.S.	15	"	11	4.6
	GP 62 ♀	None	L.S.	20	"	19	9.7
			R.S.	15	"	6†	11.2

* L.S., left side; R.S., right side.
† Some nymphs escaped on first day, as box was loose.

number and type of previous infestations and the reduction in average weight of the engorged nymphs. For example, GP 16, which had had two successive infestations with larvae and was immune to reinfestation with larvae, gave, on its first infestation with nymphs (Exper. 32), engorged ticks of almost normal average weight (9.3 mg as compared with 12.5 mg on the control). When this guinea pig was again infested with nymphs, the average weight of the engorged ticks on the two sides was only 6.7 mg and 7.5 mg (Exper. 32a), as compared with 12.7 mg and 10.7 mg on the control animal. In the same experiment, GP 24 and 29, which had had four infestations with nymphs, gave engorged nymphs which were still smaller than those from GP 16, and also smaller than

the nymphs obtained from GP 24 and 29 after only three infestations (Exper. 32). As might be expected, there was considerable variation among individual guinea pigs with respect to the degree of immunity developed. Nymphs engorging on immune guinea pigs produced much less black excreta than did those engorging on control animals, a further indication that the former take up less blood than the latter.

All the engorged nymphs were kept in small cotton-plugged test tubes over moist sand. Practically all the nymphs which had fed on control guinea pigs molted to good sized or even large vigorous adults. Most of the small nymphs engorged on immune animals molted to adults of normal appearance but of small size. Some of these nymphs gave rise to adults only 2 to 3 mm long which lacked the adult pigmentation. Superficially, they resembled overgrown nymphs. They had the same brown color as nymphs and were quite transparent. The genital pore was present, however, and males and females could be distinguished by this and by the relative size of the scutum, which, as in normal ticks, covered the dorsum of the males completely and that of the females only partially. The structures surrounding the genital pore were less developed than in normal ticks. No attempt has been made to engorge these abnormal adults, so that nothing is known of their reproductive capabilities.

The immunity of guinea pigs to adult female ticks manifested itself in much the same ways as did their immunity to the nymphs. Females which engorged on animals previously infested with nymphs or adults weighed less and produced less black excreta than control females engorging on previously uninfested animals. Some of the results with adults are given in table 6. Exper. 41 and 44 were done with reared adults, ticks entirely comparable in parentage and treatment being placed on corresponding regions of the test and control guinea pigs. In Exper. 43, wild ticks collected in Princeton were used. It will be noted that on control animals, wild females became larger than did the laboratory-reared females. Exper. 41 shows that larval infestations conferred no immunity to the adults, whereas repeated nymphal infestations, and even more, nymphal plus adult infestations, greatly reduced the average weight of the engorged females and the weight of black excreta per engorged female. Exper. 44 shows the difference between adult females engorged on litter mate guinea pigs both of which had had one larval infestation but only one of which had been previously infested by adults. The ticks on the two sides of the animals came from two different groups, so that comparison can be made only between the ticks on corresponding sides. On each side, the ticks engorged on the animal which had had one previous adult infestation were somewhat smaller and produced less excreta than those engorged on the litter mate control.

TABLE 6.—*Acquired immunity to adults of* Dermacentor variabilis

Exper. No.	Animal	Previous treatment	Region of body*	Date 4 ♀ ticks put on†	Engorged ♀ ticks		Wt. excreta per engorged ♀ tick
					No.	Av. wt.	
						mg	*mg*
41	GP 5 ♂	2 nymphal, 2 adult infestations, last on 3/28 to 4/16/38	L.S.	5/20/38	4	294	37
			R.S.	"	3	249	33
	GP 6 ♂	1 nymphal, 1 adult infestation, last on 3/28 to 4/16/38	L.S.	"	4	175	24
			R.S.	"	4	245	24
	GP 47 ♀	1 larval, 3 nymphal infestations, last on 4/26 to 5/6/38	L.S.	"	4	295	41
			R.S.	"	4	509	62
	GP 20 ♂	2 larval infestations. Immune to larvae on 12/28/37	L.S.	"	4	638	127
	GP 73 ♂	None	L.S.	"	4	556	81
43	GP 24 ♂	5 nymphal infestations, last on 4/16 to 4/26/38	L.S.	6/6/38	4	598	100
			R.S.	"	4	744	118
	GP 29 ♀	5 nymphal infestations, last on 4/16 to 4/26/38	L.S.	"	4	395	76
			R.S.	"	3	356	110
	GP 30 ♀	1 infestation with larvae and nymphs, 2 with nymphs. Last on 4/26 to 5/6/38	L.S.	"	4	632	50
			R.S.	"	4	609	92
	GP 73 ♂	1 infestation with adults on 5/20 to 5/30/38	L.S.	"	4	696	137
			R.S.	"	4	721	124
	GP 75 ♂	None	L.S.	"	4	779	158
			R.S.	"	4	818	128
	GP 76 ♂	None	L.S.	"	4	750	133
			R.S.	"	3	797	112
44	GP 20 ♂	Immune to larvae. One adult infestation on 5/20 to 5/30/38	L.S.	"	4	553	103
			R.S.	"	4	346	34
	GP 21 ♂ Litter mate of 20	1 larval infestation	L.S.	"	4	589	131
			R.S.	"	4	446	85

* L.S., left side; R.S., right side.
† An equal number of male ticks was put on with each group.

In Exper. 43, it may be seen how repeated nymphal infestations reduced the amount of blood taken by adult females. It is worthy of note that GP 29, which showed the highest degree of immunity to nymphs (Table 5, Exper. 32a), also showed the highest degree of immunity to the adults. Repeated nymphal infestations conferred more immunity to adults than one small adult infestation. It is possible that repeated heavy infestations with adults might produce a much more pronounced degree of immunity to the adults than has so far been observed. Jellison and Kohls (1938) placed successive small lots of adult *D. andersoni* on one region of rabbits and noted that toward the close of the experiment newly added ticks were unable to attach on the encrusted area, while those which were attached fed slowly. Both of these phenomena may have been simply the result of the encrustation, or the immunity here reported may also have played a part. It should be emphasized that in

the experiments with nymphs and adults detailed in the present paper sufficiently small numbers were used and enough time was allowed to pass between infestations so that, at the time of reinfestation, the scabs formed from the previous infestation were almost or completely healed and covered with new growth of hair. Moreover, in some experiments the immunizing infestation was applied on one side of the body, and the test dose on both sides.

III. *Artificial and Passive Immunization.* In these studies, only the larvae of *D. variabilis* were used, as they furnished the best and most convenient test organism.

Several experiments, two of which are given in table 7, have shown that the acquired immunity of guinea pigs can be artificially produced by the intracutaneous inoculation of an extract of larval ticks. The extract used for Exper. 24 was prepared by grinding approximately 400 larvae in 0.85% sodium chloride solution, centrifuging and diluting the supernatant with the saline solution to 8 cc. That used for Exper. 34 was prepared by grinding about 500 larvae in saline solution, centrifuging and diluting the supernatant to 6 cc. Both extracts were slightly yellowish and opalescent. As is clearly shown in table 7, a series of injections on the left side only with either of these extracts produced a rather solid immunity on both sides and a partial immunity on the ear. The control guinea pigs were inoculated with saline solution, except for GP 59 which received an extract of 7 adult female *Aedes aegypti* mosquitoes in 6 cc of saline solution. As indicated in table 7, guinea pigs receiving tick extract developed small, circular, reddish, hardened, depilated areas at the site of injection.

It has been possible to transfer the immunity passively by the intraperitoneal inoculation of serum from "hyperimmune" animals (Table 8). Guinea pigs which had been repeatedly infested with nymphs, such as GP 24, 29, 30 (Table 5) were considered as having been hyperimmunized and were the serum donors for the passive immunization experiments. Blood was obtained by heart puncture. The first inoculation with serum was always performed immediately after the application of the ticks. As shown in table 8, the immunity conferred by passive transfer was usually not a very solid one, but it consistently reduced the number of larvae able to engorge by about 50%. In Exper. 40, one inoculation of 1 cc of serum per 100 gm body weight produced as much immunity as did two inoculations of 1 cc or of 1.25 cc. Some of the larvae which engorged on guinea pigs receiving immune serum were pale brown rather than black. Such pale brown larvae were frequently obtained from previously infested hosts (see remarks, Table 1, and section IV of Results), but out of the hundreds of larvae engorged on control guinea pigs all were black except one. These facts furnish

TABLE 7.—*Artificial immunization to larvae of* Dermacentor variabilis

Exper. No.	Animal	Treatment	Region of body*	Ticks put on		No. engorged	Remarks
				No.	Date		
24	GP 44 ♀	Injected intracutaneously on left side with larval tick extract—0.05 cc on 3/2/38, 0.1 cc on each of 3/3, 3/4, 3/5, and 3/7	L.S.	100	3/10/38	0	
			R.S.	100	"	1	Brown and undersized
	GP 45 ♀ Litter mate of 44	Injected like 44 but with 0.85% NaCl solution	L.S.	100	"	33	
			R.S.	100	"	25	
34	GP 56 ♂	Injected intracutaneously on left side with 0.1 cc of larval tick extract on 4/2, 4/4, 4/5 4/6, 4/7/38	L.S.	100	4/14/38	6	On 4/5 shows reddish hardened region at site of injections
			R.S.	100	"	13	On 4/14 only slight scabs remain
			L.E.	100	"	26	1 of engorged larvae brown
	GP 57 ♂ Litter mate of 56	Injected like 56, but with 0.85% NaCl solution	L.S.	100	"	42	No reaction on skin
			R.S.	100	"	41	
			L.E.	100	"	44	
	GP 58 ♂	Injected exactly like 56	L.S.	100	"	0	On 4/14 shows 3 marked red depilated circular areas at site of injection
			R.S.	100	"	1	
			L.E.	100	"	51	
	GP 59 ♂ Litter mate of 58	Injected like 56 but with extract of adult ♀ *Aedes aegypti*	L.S.	100	"	35	No reaction on skin
			R.S.	100	"	11	
			L.E.	100	"	72	

* L.S., left side; R.S., right side; L.E., left ear.

further evidence for the transfer of an antibody in the immune serums. The time relation between the application of larvae and the inoculation of serum eliminates the possibility of the immunization having been the result of a transfer of circulating antigen, as in the experiments of Stumberg (1933) on the nematode *Haemonchus contortus*. Moreover, when the donor guinea pigs were bled no ticks were attached to them, so that it was very unlikely that tick antigen was still present in the blood.

A number of attempts to demonstrate antibodies *in vitro* in serums successfully used for passive immunization, failed. Extracts of tick larvae in 0.85% sodium chloride solution were used as antigens. In one case a very faint ring precipitin test was obtained. The complement fixation test could not be used because it was found that normal inactivated guinea pig serum, at dilutions up to 1:32, reacted with the tick extract to fix complement. The tick extract at the concentration used

TABLE 8.—*The passive transfer of immunity to larvae of* Dermacentor variabilis

Exper. No.	Animal	Treatment	Region of body*	Ticks put on		No. engorged	Remarks
				No.	Date		
23	GP 42 ♀	Inoculated intraperitoneally with serum from immune guinea pigs, receiving per 100 gm wt. – 0.4 cc on 3/1, 0.8 cc on 3/2, 0.9 cc on 3/3 and 1.1 cc on 3/4/38	L.S.	100	3/1/38	2	
			R.S.	100	"	1	This one of a light brown color
	GP 43 ♀ Litter mate of 42	None	L.S.	100	"	30	
			R.S.	100	"	10	
31	GP 53 ♀	Inoculated intraperitoneally with serum from immune guinea pigs, receiving per 100 gm wt. 1 cc on 3/17 and 1.5 cc on 3/18, 3/19, 3/20/38	L.S.	100	3/17/38	14	
			R.S.	100	"	25	
			L.E.	100	"	7	Total = 46
	GP 54 ♀ Litter mate of 53	Inoculated like 53 but with serum from normal guinea pigs	L.S.	100	"	18	
			R.S.	100	"	42	
			L.E.	100	"	26	Total = 86
40	GP 69 ♂	Inoculated intraperitoneally with immune GP serum, receiving per 100 gm wt. 1 cc on 5/12/38	L.S.	100	5/12/38	13	
			R.S.	100	"	8	
			L.E.	100	"	66	Total = 87
	GP 70 ♂ Litter mate of 69	Inoculated intraperitoneally with immune GP serum, receiving per 100 gm wt. 1 cc on 5/12 and on 5/14/38	L.S.	100	"	15	Total = 92. One from ear was pale brown in color
			R.S.	100	"	5	
			L.E.	100	"	72	
	GP 71 ♂ Litter mate of 69	Inoculated intraperitoneally with immune GP serum, receiving per 100 gm wt. 1.25 cc on 5/12 and on 5/14/38	L.S.	100	"	20	Total = 88. About half of those from ear were pale brown in color
			R.S.	100	"	15	
			L.E.	100	"	53	
	GP 72 ♂ Litter mate of 69	Inoculated like 71 but with normal GP serum	L.S.	100	"	46	
			R.S.	100	"	41	
			L.E.	100	"	76	Total = 163

* L.S., left side; R.S., right side; L.E., left ear.

had no anticomplementary or hemolytic action. Neither the normal nor the immune guinea pig serum at the dilutions of 1:8 and higher had any anticomplementary effect. But normal serum plus tick extract fixed complement as much as did immune serum plus tick extract. Preliminary work indicates that rabbit serum will not show such a nonspecific complement fixation with tick extract, in which case it may be possible to demonstrate specific complement fixation with serum from immune rabbits.

IV. *The Cellular Reaction in Non-Immune and Immune Guinea Pigs.* The bites of adult ticks generally produce edematous inflammatory lesions (Hooker, Bishopp and Wood, 1912). Wolbach (1919) has described the histology of the adult tick bite. The epidermis at the point of attachment is absent and the surface of the exposed corium is necrotic and infiltrated with fibrin and leucocytes. The corium is edematous, and new fibroblasts and mononuclear phagocytic cells are present in large numbers. Gregson (1937) has recently studied the histology of adult tick bites on sheep and has also observed an acute edematous inflammation at the site of attachment. Talice (1930) has described the appearance of sections through the points of attachment of larvae of *Dermacentor reticulatus* on the ear of a hedgehog. His photomicrographs show some thickening of the cornified layer of skin and some inflammatory reaction with a hemorrhagic region at its center. The epithelium is lacking at the site of attachment and is not thickened at the edges of the bite.

In an attempt to discover some visible reaction which could account, at least in part, for the acquired immunity of guinea pigs to infestation by larvae of *D. variabilis,* a study was made of the histology of larval tick bites on the ears of susceptible and partially immune animals. On the 4th to 6th day after the application of the ticks, a small portion of ear bearing attached larvae was cut off and fixed in Carnoy-Lebrun. In order to improve the quality of the sections through the chitinous parts of the ticks, a method described by Slifer and King (1933) for grasshopper eggs was used. After fixation and treatment with iodine in 70% alcohol, the tissues were left one day in 80% alcohol containing 4% phenol. They were then passed through 95% alcohol, aniline oil, xylene, and embedded in paraffin in the usual manner. The paraffin blocks were cut enough to expose the tissue and were then soaked overnight in water. Serial sections of entire tick larvae, nymphs and even adult, recently molted females could be obtained in this way. The sections of the ears and attached larvae were stained by Wolbach's (1919) Giemsa method. Plate II, Fig. 1, is typical of the appearance of sections through the point of attachment of a tick larva on a susceptible guinea pig. This ear was fixed on the 4th day after the application of the ticks, when the attached larvae were almost fully engorged. The epithelium is lacking at the point of attachment. The mouthparts are surrounded by a mass of pink-staining material, presumably fibrin. Beneath them there is generally a small hemorrhagic area but practically no cellular reaction. The epithelium is not thickened and there is no leucocytic infiltration. Plate II, Fig. 2, shows a section through the right ear, fixed on the 4th day after application of the ticks, of a guinea pig previously infested on its left ear only (see Table 1, Exper. 17, GP 33). On the first day after

application, numerous attached larvae were noted on this ear, but by the 4th day there were relatively few and these were only partly engorged. A study of a series of sections through the still infested piece of ear has shown reactions of which that pictured in the photomicrograph is entirely typical. The pink-stained mass of fibrin is surrounded by a large inflammatory reaction. The solid mass of leucocytes contains all types of cells, but polymorphonuclears and small polyblasts are especially numerous. There are very few eosinophiles. The epithelium at the edges of the bite is thickened and has grown down beneath the leucocytic mass. The whole region is edematous. Thus, by the 4th day, before the tick larva has been able to engorge, it is effectively walled off from its source of supply of food. This same type of reaction has been seen in sections made from a number of partially immune guinea pigs studied in this manner. Frequently, the reaction is macroscopically visible as a white blister. The intensity of the reaction varies considerably, especially with respect to the degree of epithelial growth. This variation may be due to variations among the ticks as well as among the guinea pigs. On one ear of a partially immune guinea pig two ticks were attached side by side. Beneath the mouthparts of one there was a relatively small leucocytic mass and no epithelial thickening. Beneath the mouthparts of the other there was a large leucocytic mass and the epithelium was thickened and had started to grow down. The former tick had a normal black color at the time the ear was fixed, while the latter tick was a very pale brown.

Attention has already been drawn to the abnormally colored tick larvae so frequently obtained from partially immune hosts. Plate I, Fig. 4, shows a normal, black, engorged larva and one of the very pale brown, almost white larvae. The color of these pale larvae is undoubtedly the result of the cellular reactions just described. Such larvae suck up leucocytes instead of red blood cells. This fact has been confirmed in sections of normal and white engorged larvae. The alimentary tract cells of normal larvae appear very large and full of irregular deep-pink-stained globules. In the lumen of the gut there is a solid dark-rose-colored mass with a few intact red cells and a few leucocytes. There are also many brown granules resulting from the digestion of hemoglobin. In an exactly comparable pale individual, the alimentary tract cells appear small and some distance away from the hypodermis. The lumen of the gut is filled with a diffuse granular light blue staining material containing many leucocytes. Some of the pale engorged larvae successfully molted to decidedly undersized nymphs, but many of them died, although kept under the same conditions as normal engorged larvae, almost all of which molted.

In addition to the very pale larvae and the larvae of various shades of brown, representing gradations between the former and normal larvae, there were occasionally obtained, from immune guinea pigs only, bright red engorged larvae and reddish brown ones. The bright red ones always died soon after detaching. At a stage when the gut contents of normal larvae, examined in the fresh state, contained some large hemoglobin crystals and many black granules, the gut contents of the red larvae contained intact red blood cells and no black granules. It would seem that something had interfered with the normal digestive processes of the tick.

Only one experiment concerned with the cellular reaction to nymphal tick bites has been performed. Two litter mate female guinea pigs were used, one of which was infested with larvae of *D. variabilis* on both sides and both ears. Six days later, 12 nymphs of *D. variabilis* were placed on the right ear of each. Eight days still later the last nymphs to engorge had dropped from both animals. Nine engorged nymphs were obtained from the previously infested one and 12 from the litter mate. The former ticks averaged slightly less in weight than the latter. The right ear of each animal showed small white blisters at the points where the ticks had been attached. Two such regions from the ear of each animal were fixed and sectioned. Sections of both blisters from the previously uninfested animal appeared as shown in Plate III, Fig. 1. The mass of fibrin is continuous with a considerable hemorrhagic region, the epithelium is somewhat thickened, and there is a pronounced inflammatory edema. The picture is very similar to that described by others for adult tick bites. Plate III, Fig. 2, shows the appearance of sections through both blisters from the previously infested guinea pig. Beneath the fibrin is an enormous leucocytic mass (cracked in the process of sectioning) which is already walled off by a new growth of epithelium. Although the one previous infestation with larvae had almost no effect on the percentage of nymphs engorged or their weight, it did greatly accelerate the foreign body reaction around the site of attachment. Probably, in animals repeatedly infested with nymphs, this reaction is still more rapid and extensive, accounting for the abnormally small size of nymphs engorged on such animals.

DISCUSSION

I. An effective acquired immunity to larvae of the tick, *Dermacentor variabilis,* can be induced in guinea pigs by a single infestation with larvae or nymphs. This immunity develops to its fullest extent within about two weeks after the beginning of the first infestation and lasts at least three months. The immunity is a general one. A local immunity, however, also plays some part, as during the course of establishment of the

immunity, regions which have been infested by ticks show a greater resistance than previously uninfested regions. The immunity can be passively transferred by the inoculation of serum, showing that circulating antibodies are concerned in the immune mechanism. On the basis of the cellular reactions in non-immune and immune guinea pigs, it seems reasonable to conclude that the circulating antibodies greatly accelerate and intensify the foreign body reaction at the site of attachment of the larval tick and so wall it off from its blood supply before it can become engorged. Repeatedly, I have observed a mass of larvae attach on the ear of an immune guinea pig and begin to engorge. By the third or fourth day, however, a large blister would be present at the region of attachment of the ticks. Most of the ticks would then gradually shrivel and die while still attached. Generally, a few small pale-colored ones would detach themselves and survive. It is possible that there is also some immune mechanism in addition to this accelerated and intensified foreign body reaction. On very solidly immune guinea pigs, much fewer larvae appeared to attach in the first place than on partially immune or susceptible animals. Since observations were not made at intervals of less than one day, perhaps the larvae did attach and then quickly detached. At any rate, the cellular reaction itself can account for the observed completely effective immunity to the larvae and for the reduction in the amount of blood taken by nymphs and adults from immune animals.

The acquired immunity to ticks thus involves essentially the same mechanism which is apparently concerned in all known immunity to metazoan parasites and possibly to all infectious agents—an antibody accelerating a local reaction in strategically placed organs (Taliaferro, 1934). The cellular reactions of immune animals to certain helminth parasites provide several interesting similarities to the reactions at the tick bites. The experiments of Sarles and Taliaferro (1936) and Taliaferro and Sarles (1937) with the nematode *Nippostrongylus muris* show that, whereas in the skin of normal rats migrating larvae incite a mild temporary diffuse inflammation, in the skin (and lungs) of immune rats they are temporarily immobilized and are surrounded by cell accumulations. The immunity is passively transferable but acts locally in certain organs. Turner, Dennis and Berberian (1937) have found that in sheep previously injected with hydatid antigen the cysts of *Echinococcus granulosus* are effectively walled off and are much smaller than in untreated animals. Kerr (1938a) has likewise observed the rapid walling off of pig ascaris larvae in immunized guinea pigs and has obtained some evidence (1938b) for the presence of circulating antibodies.

II. The question as to what is the actual antigenic substance injected by the attached ticks is of considerable interest. Since the tick's mouthparts are the only portion of it coming in contact with the host's tissues, it seems very probable that the antigenic substance is injected during the process of feeding and is some material present in the salivary secretion. Nuttall and Strickland (1908), Cornwall and Patton (1914), Khodukin and Sofiev (1931) and Hoeppli and Feng (1933) have all demonstrated the presence of powerful anticoagulins in the salivary glands of ticks. Possibly the anticoagulin functions as antigen. Cornwall and Patton (1914), however, could not induce in rats or rabbits the formation of an antienzyme to the anticoagulin of the fly *Philaematomyia insignis*.

There is some evidence that the salivary secretions of blood-sucking insects act as antigens. Boycott (1926, 1928) and Peacock (1926) report observations indicating that the wheal, resulting from the bite of certain insects on susceptible persons, forms only if the person has been previously bitten by the same kind of insect. The first bite produces a sensitization and the wheal following subsequent bites is in the nature of an allergic reaction. Lester and Lloyd (1928) have shown that tsetse-flies from which the salivary glands have been removed are able to live and take blood meals for some time but produce no skin reaction even in very susceptible persons.

III. It has long been popularly held that the natives of regions heavily infested by blood-sucking arthropods are not bothered by them nearly as much as newcomers. Nuttall and Strickland (1908) state that strangers to a district suffer more severely than natives from tick and mosquito bites. The question still remains: are the resistant natives not troubled by the arthropod because it does not bite them, or because, although they are bitten as frequently as non-resistant strangers, they do not react to the bites? Either or both phenomena may well occur with different species of arthropods. Rozeboom (1936) remarks that the donor of blood to a laboratory colony of *Anopheles albimanus* soon became immune to the effects of the mosquito bites, although the insects presumably continued to feed on him as well as ever.

According to Ross (1926), there is evidence that dogs living for many years in tick-infested places in Australia rarely suffer from tick paralysis. Tzortzakis and Papadakis (1936) similarly note that in Crete young sheep and goats are much more susceptible to tick paralysis than older ones. The field observations of Johnston and Bancroft (1918) on a tick-resistant condition of certain cattle in Queensland, Australia, furnish the only previously reported evidence which indicates the possibility of an acquired resistance to ticks themselves. Johnston and Bancroft repeatedly observed that cows of different breeds which were

heavily infested with *Boophilus australis* Fuller during one year in the paddock, or were allowed to run wild (and hence probably heavily infested also), were subsequently resistant. Larval ticks would attach, but few were able to mature. The resistant cows frequently showed a yellow exudate on various parts of the skin during the tick season and formed, at the site of attachment of a tick, a peculiar scab never seen on susceptible animals. The tick attached at the region of such a scab dried up and was shed with the scab. The similarity between this reaction and that in guinea pigs immune to *D. variabilis* suggests that the resistance of cattle to *B. australis* observed by Johnston and Bancroft was an acquired immunity of the same type as that exhibited by guinea pigs to *D. variabilis*. On the other hand, Legg (1930) did not observe any increased resistance of cattle to the cattle tick *Boophilus australis* following prolonged exposure.

Most of the work on immunity to metazoan parasites, beginning with that of Sandground (1928) and Stoll (1929), has been concerned with helminth parasites (see Culbertson, 1938, for a review of the literature). But as long ago as 1919 Reuling showed that fish could acquire immunity to the glochidia of fresh water mussels. Blacklock and Gordon (1927) and Blacklock, Gordon and Fine (1930), in one of the earliest detailed studies on immunity to metazoan parasites, found that guinea pigs acquired immunity to larvae of the fly *Cordylobia anthropophaga*. These larvae burrow in the subcutaneous tissues of their host. The only ectoparasites, other than ticks, to which the development of an acquired immunity has been demonstrated are the glochidia already mentioned and *Epibdella mellani*, a trematode parasite of marine fish (Nigrelli and Breder, 1934). The present paper furnishes the first experimental evidence for the development of a true acquired immunity to a blood-sucking arthropod. It thus extends to yet another group of parasites the classical concepts of immunology which, during the past ten years, have been shown to apply as much to metazoan as to bacterial and protozoan parasites.

SUMMARY

One infestation of guinea pigs or rabbits with larvae of the American dog tick, *Dermacentor variabilis*, induces an acquired immunity which effectively prevents subsequent batches of larvae from engorging. In guinea pigs, the immunity develops fully within two weeks after the start of the first infestation and lasts at least three months. Guinea pigs first infested with either *D. variabilis* or *D. andersoni* show a cross immunity to larvae of the other species. Similarly, rabbits first infested with either *D. variabilis* or *Haemaphysalis leporis palustris* show cross

immunity to larvae of the other species. Deer mice become relatively resistant to larvae of *D. variabilis* after two or three infestations.

The repeated infestation of guinea pigs with nymphs or adults of *D. variabilis* results in a marked reduction in the amount of blood taken by ticks of the later batches.

The immunity of guinea pigs to larvae of *D. variabilis* can be produced artificially by the intracutaneous inoculation of an extract of larval ticks. It can be passively transferred by the intraperitoneal inoculation of serum from guinea pigs hyperimmunized by repeated infestations with nymphs.

In the ears of non-immune guinea pigs, on the fourth day after attachment of a larva of *D. variabilis*, there is little cellular reaction at the site of attachment. In immune animals, there is present by the fourth day an intense inflammatory reaction. The mouthparts of the tick are met by a solid mass of leucocytes and the epithelium has thickened and begun to grow beneath the leucocytic mass. In this way, the tick becomes walled off from its source of supply of blood before it can engorge.

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PLATE I

Photographs by J. Carlile.

FIG. 1. Guinea pig with pill boxes attached on sides and over ears.

FIG. 2. Deer mouse 2 on the fifth day of its fourth infestation with larvae of *D. variabilis*.

FIG. 3. Deer mouse 6 on the fifth day of its first infestation with larvae of *D. variabilis*.

FIG. 4. Normal engorged larva of *D. variabilis* (left) and a very pale brown engorged larva (right). ×17.

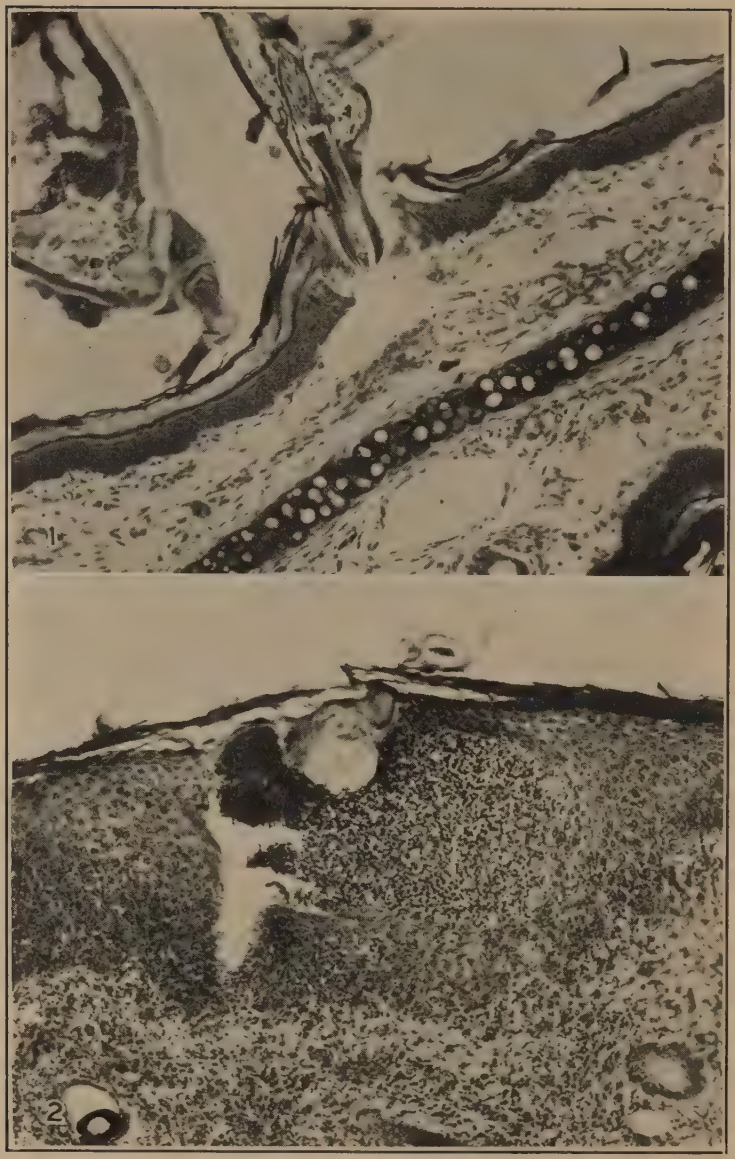

PLATE II

Sections through the point of attachment of a larva of *D. variabilis* on the ear of a susceptible guinea pig (Fig. 1), and an immune guinea pig (Fig. 2). Both × 150. Giemsa stain. (Photographs by J. Carlile.)

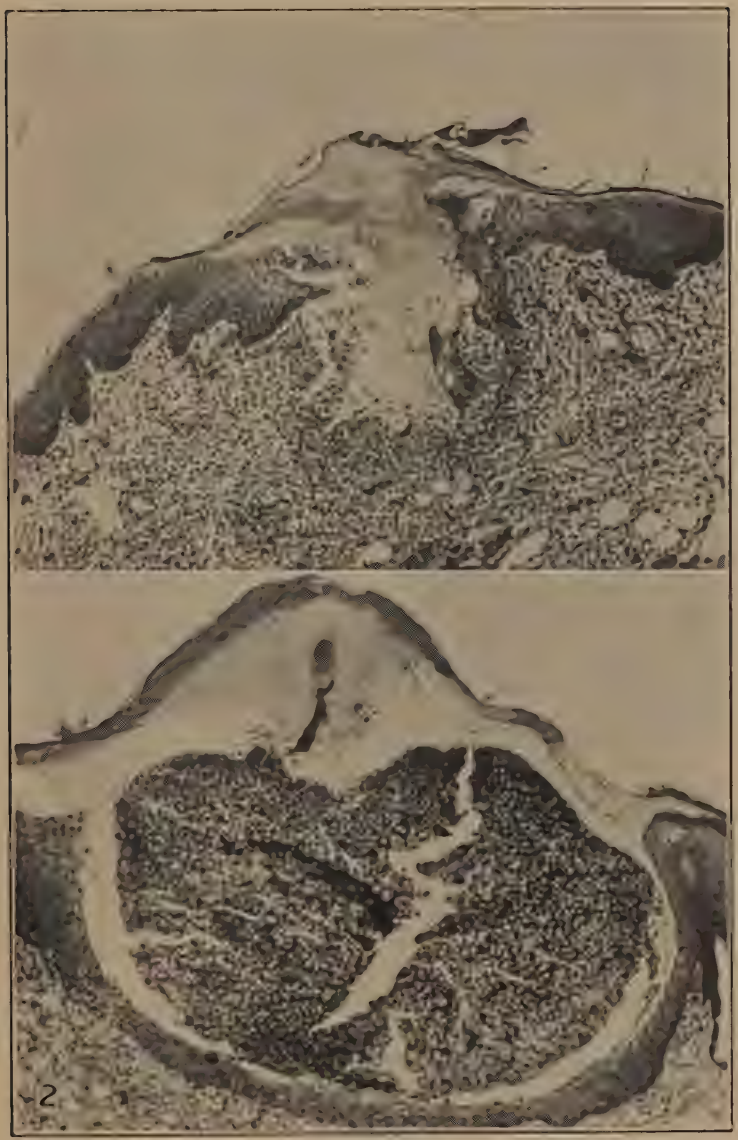

PLATE III

Section through a nymphal tick bite on the ear of a previously uninfested guinea pig (Fig. 1) and a guinea pig previously infested with larvae (Fig. 2). Both ×97. Giemsa. (Photographs by J. Carlile.)

STUDIES ON *LOPHOTASPIS VALLEI* (STOSSICH, 1899) (TREMATODA: ASPIDOGASTRIDAE)

G. W. Wharton
Duke University

A two hundred pound loggerhead turtle, *Caretta caretta* (L.), was captured near Indian Pass, Gulf Co., Florida, July 22, 1935. It was examined for parasites and yielded among other things, two adult specimens of *Lophotaspis vallei* from the stomach. Several months later, Mr. Gorden Gunter collected some large specimens of the flag conch, *Fasciolaria gigas* (L.), ten miles west of the spot where the turtle was captured. He dissected some of these to study the anatomy of the radula (Gunter, 1936) and found six young aspidogastrids.

The material from the turtle and conch was fixed in Conant's solution, stained in borax-carmine, and mounted in gum damar. A comparison of the specimens shows that the former is the adult stage of the latter; they differ only in size and in the degree of development of the sexual organs. The adult was described by Looss (1902), but the nymph is reported here for the first time.

DESCRIPTION OF NYMPH

General shape like that of adult. Oral cavity 0.5 mm long. Oval pharynx, 0.6 mm long, leads into blind sac-like intestine, 4.5 mm long. Genital pore just ventral to oral opening. Vas deferens, an undulating tube, extends from genital pore to the small testis, 0.2 mm in diameter. Slightly coiled uterus lies ventral to gut and extends from pore to oötype, 0.1 mm in diameter, which is just dorsal to testis. Three other ducts enter oötype. Yolk duct enters on ventral side, while oviduct and Laurer's canal enter on dorsal side. Ovary, 0.15 mm in diameter, is 0.2 mm in front of oötype. Laurer's canal opens on dorsal surface just above oötype. Yolk glands consist of small follicles which extend from middorsal region of body to posterior margin. Excretory bladder notched anteriorly. Ventral sucker characteristic of the genus, having papillae at junction of each three alveoli, except at extreme anterior and posterior ends where they are more numerous; there are 81 and 79 alveoli in the two available specimens.

DISCUSSION

Ward and Hopkins (1931) described a new species of *Lophotaspis* from an alligator snapper *Macrochelys temminckii* (Holbrook) taken near Cord, Arkansas, which they named *L. interiora*. The single specimen was immature; it contained but three eggs in the uterus. They compared their worm with *L. vallei* and found the following differences:

L. interiora is much smaller both in total body size and in the dimensions of the ventral disc. *L. vallei* has 74 to 77 alveoli, *L. interiora* only 65. *L. vallei* has an undivided excretory bladder, while in *L. interiora* the excretory bladder is

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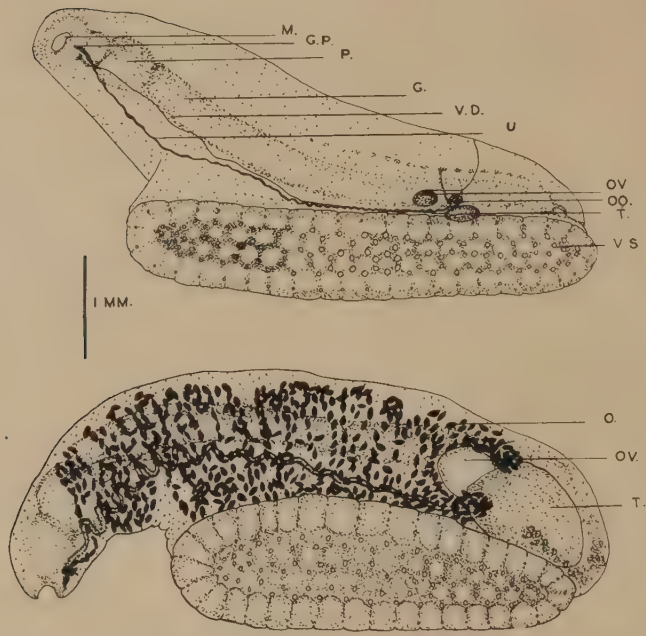

FIG. 1. *Lophotaspis vallei* (Stossich, 1899). Nymph above, adult below.

Abbreviations: G., gut; G.P., genital pore; M., mouth; O., ovum in uterus; OO., oötype; OV., ovary; P., pharynx; T., testis; U., uterus; V.D., vas deferens; V.S., ventral sucker.

deeply bifid. In *L. vallei,* the pharynx is much larger than the oral cavity and is much longer than it is wide, while in *L. interiora* the pharynx is approximately the same size as the oral cavity and nearly spherical. *L. vallei* occurs in marine turtles of the Mediterranean Sea, while *L. interiora* was found in a fresh water turtle of the Mississippi Valley.

The first difference between *L. interiora* and *L. vallei,* that of size, is not as significant as it may seem. My immature specimens are extended and, although only about half the volume of the mature specimens, equal them in length. Looss (1902) gives the length of *L. vallei* as 9 to 12 mm. The writer's adult specimens are 5 and 6 mm in length, while the immature specimens are 5 mm in length. Although *L. interiora* is only 3.9 mm in length, it is an immature specimen from an unusual host (if *L. interiora* be synonymous with *L. vallei,* as seems to be the case). In a contracted state, the 5 mm specimens from the snail would surely be less in length than 3.9 mm.

L. vallei has 74 alveoli (Stossich, after Looss, 1902), 77 (Looss, 1902), and from 69 to 82 in the writer's specimens; *L. interiora* is said to have 65. This difference of 4 is not very significant as the range above

69 is 13. The immature specimens of *L. vallei* have a deeply bifid excretory bladder, thus agreeing with *L. interiora*. In the contracted adult specimens of *L. vallei*, the pharynx is subspherical and about the same length as the oral cavity, thus agreeing with *L. interiora*, which, as predicted, is likewise somewhat contracted.

L. vallei is present in the Gulf of Mexico (Manter, 1932) and is thus not so far removed from the locality in which the host of *L. interiora* was found. However, it is not easy to explain the presence of a marine trematode, 700 miles inland in an unusual host. Ward and Hopkins state that although the chance is remote, one must keep in mind the possibility that this snapping turtle, while in captivity, may have been fed some marine oysters from the Gulf and that the parasite was a marine species thus introduced adventitiously. It seems even more improbable that a conch the size of *Fasciolaria gigas*, would be fed to a turtle so far inland. Although the worm might have been in the oyster, and Shipley and Howell (1904) reported a related species from the pearl oyster, the author feels that this is highly improbable, since he examined thousands of oysters in the locality from which he obtained *L. vallei* and never found them infected with this conspicuous worm. In spite of the fact that the worm has been found in a fresh-water snapping turtle, the author believes the two species to be identical, although there is still a reasonable doubt as to the synonymy of *L. vallei* and *L. interiora*.

Faust and Tang (1936) have described a valid species of *Lophotaspis*, *L. orientalis*, from a fresh-water turtle in China, where it is apparently not rare. This lends support to the discovery of Ward and Hopkins. The only other *Lophotaspis* recorded is *L. macdonaldi* (Monticelli), which is apparently an immature form from *Melo* sp., a marine gastropod from Australia. This is probably a valid species, since it has 120 alveoli (Ward and Hopkins, 1931) as compared to 82 in *L. vallei* and less in other species.

LIFE HISTORY OF *Lophotaspis vallei* (STOSSICH, 1899)

L. vallei hatches from the egg as an elongated oval larva from 0.15 to 0.20 mm in length. It has eye spots, oral and posterior suckers and three patches of cilia. It swims about with its cilia or crawls like a leech (Manter, 1932). In this way, it would easily be able to migrate to the mantle cavity of its snail host, *Fasciolaria gigas*. The snails are probably eaten by the turtles to complete the cycle. The author has seen turtles pick up conchs as though they were about to eat them, but has never seen one actually eaten.

This life history is particularly significant because it is a connecting link in the course of evolution of the trematodes. Some aspidogastrids become mature in molluscan hosts; others in vertebrates by way of mol-

luscan hosts. This fact supports the theory that the trematodes were originally adapted to live parasitically in molluscs and later became parasites of vertebrates while still retaining their ancestral hosts in the developmental stages.

SUMMARY

1. The nymph of *Lophotaspis vallei* is described from the snail *Fasciolaria gigas*.

2. *L. interiora* Ward and Hopkins, 1931, is very close to, if not synonymous with *L. vallei*.

3. *L. vallei* is adult in the turtle; the larva is free-swimming; and the nymph occurs in at least one species of snail, *Fasciolaria gigas*.

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CERCARIA PSEUDOBURTI N. SP., A STRIGEID CERCARIA FROM WESTERN MASSACHUSETTS

JOHN S. RANKIN, JR.

Amherst College

A survey of many ponds in western Massachusetts has revealed a high percentage of infection with larval trematodes. Collections are being made in some of these ponds every month to study the ecology of the cercarial fauna. Over 3,000 snails have been taken from a small pond in South Amherst, only one of which has been infected with a small species of strigeid cercaria. Since this cercaria has occurred but once, and since as far as can be determined it is specifically different from other known species, it is described here in detail as *Cercaria pseudoburti* n. sp.

METHODS OF STUDY

Snails were isolated in half pint bottles and examined regularly for escaping cercariae. All morphological details were studied on living specimens. Since only one snail was infected and infection experiments were being attempted, studies of immature stages were not carried out. These cercariae are so transparent, however, that all structural details could be observed on mature individuals. Several intra-vitam stains were tried but none were of much advantage. The use of dilute urine aided in determination of excretory tubules. Measurements were made on both living and preserved specimens (Table 1). Cercariae were killed by anesthetizing with neutral red and then plunging into hot 10 per cent formalin, stained with precipitated borax carmine, and mounted in damar. Uniform fixation was obtained with this procedure with practically no distortion.

Cercaria pseudoburti n. sp.

(Figs. 1–3)

Specific diagnosis: Strigeid cercaria; small form with body in living specimens somewhat longer in length and width than the tail stem; furcae longer than tail stem; in constant motion when free in water, occasionally resting on bottom; well-developed acetabulum and pharynx; intestinal ceca extend to posterior edge of acetabulum; eight penetration glands, small, in two groups of four, between acetabulum and genital primordium; caudal bodies large, usually seven pairs, with a pair at base of furcae; body spines limited to anterior half of anterior organ; excretory system with post-acetabular commissure; six flame cells on each side in body, one pair in anterior tail stem; two small, lateral, unpigmented eyespots between pharynx and acetabulum.

Host: Lymnaea (*Pseudosuccinea*) *columella* Say.

Locality: South Amherst, Massachusetts.

Type and cotype specimens: U. S. Nat. Mus. Helm. Coll.

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Distribution. *Cercaria pseudoburti* has been found in only one pond of the many examined during the period September, 1936, to February, 1938. Only one snail of over 3,000 examined, moreover, was infected with this species. It is considered as an occasional parasite in this region,

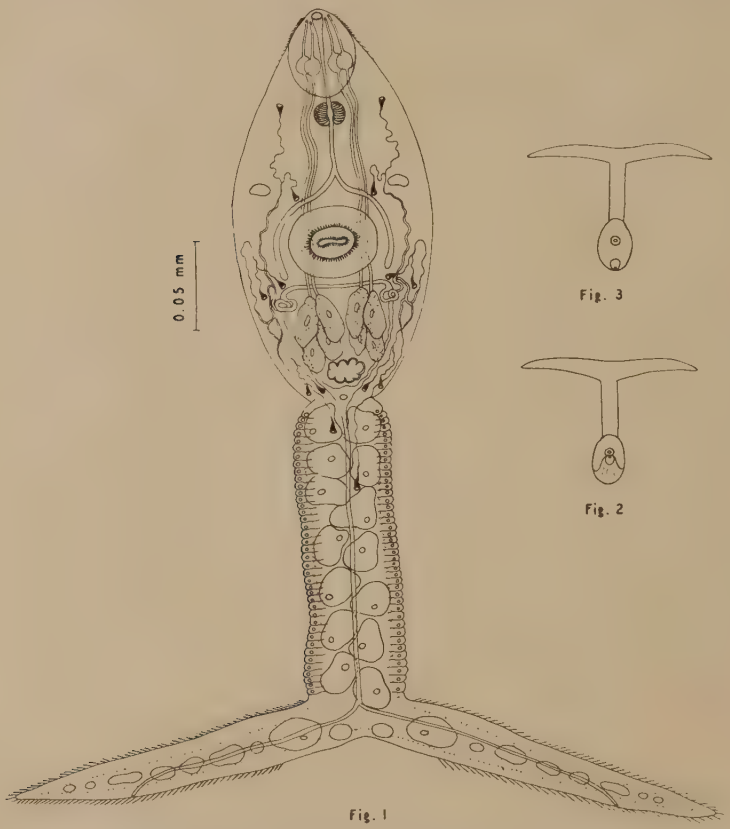

FIG. 1. Camera lucida drawing of living specimen of *C. pseudoburti,* showing body details and arrangement of the excretory system.
FIG. 2. Sketch of *C. pseudoburti* floating.
FIG. 3. Sketch of *C. pseudoburti* at rest on bottom.

probably mature in a migratory host. The infected snail collected in October, 1937, was shedding about 500–700 cercariae per day, and continued shedding until it died on January 25, 1938.

Activity. Simple experiments showed that *C. pseudoburti* is negatively phototropic. Cercariae emerge primarily during the night, very few during the day. They swim constantly, tail foremost, often chang-

ing their direction. At times, many have been observed suspended motionless in the water (Fig. 2) or quiet on the bottom (Fig. 3), which resume swimming at the slightest stimulus. There is a tendency for them to collect near the top of the water opposite the light.

Size and shape. Measurements in millimeters for both living and preserved specimens are presented in Table 1. Length and width of

TABLE 1.—*Measurements in millimeters of ten living and ten preserved specimens of* Cercaria pseudoburti

	Living			Preserved		
	Max.	Min.	Ave.	Max.	Min.	Ave.
Body length extended	0.21	0.17	0.19	0.14	0.10	0.11
Body length contracted	0.09	0.07	0.08			
Body width extended	0.03	0.03	0.03	0.05	0.04	0.04
Body width contracted	0.13	0.09	0.10			
Tail stem length	0.18	0.14	0.15	0.15	0.14	0.14
Tail stem width	0.06	0.04	0.05	0.04	0.03	0.03
Furcae length	0.20	0.17	0.17	0.17	0.14	0.16
Furcae width	0.03	0.01	0.02	0.01	0.01	0.01
Anterior organ length	0.05	0.04	0.04	0.04	0.03	0.03
Anterior organ width	0.05	0.04	0.05	0.03	0.02	0.03
Pharynx length	0.02	0.01	0.01	0.01	0.01	0.01
Pharynx width	0.02	0.01	0.02	0.01	0.01	0.01
Acetabulum length	0.05	0.04	0.04	0.04	0.03	0.04
Acetabulum width	0.05	0.04	0.04	0.04	0.03	0.03
Eyespot length	0.01	0.01	0.01			
Eyespot width	0.01	0.01	0.01			

body tends to be somewhat more than that of tail stem. Furcae are slightly longer than tail stem. The anterior organ and acetabulum are of about equal size, the latter located just posterior to the middle of the body.

Eyespots. A characteristic feature of *C. pseudoburti* is the presence of two small bodies lateral and anterior to the acetabulum. These are clear, opaque areas and no internal structure could be observed. Although no suggestion as to their function can be made, it is apparent that they are similar to the "unpigmented eyespots" of other strigeid cercariae and, except for size, resemble those of *C. ranae* Cort and Brackett, 1938.

Spination. A small area of closely set fine spines extends from the oral aperture over the anterior three-fourths of the anterior organ. Two rows of fairly large, irregularly-placed spines surround the opening of the acetabulum. The tail stem is devoid of spines but the whole anterior and three-fourths of the posterior edge of the furcae bear long slender spines.

Digestive tract. The mouth is subterminal, opening on the ventral surface. A short prepharynx is followed by a large, muscular pharynx. The long esophagus forks at about the level of the eyespots and the two ceca extend laterally around the acetabulum to the posterior edge of this sucker.

Penetration glands. Eight penetration glands in two groups of four each are located posterior to the acetabulum, between it and the genital primordium. Each gland is oval in outline with a large distinct nucleus

and is very glandular. Four ducts, two on each side, extend dorsal to the acetabulum, lateral to the pharynx, and pass through the anterior organ to open beside the mouth. A striking feature of this species is the inflation of the four ducts in the anterior organ as indicated in Fig. 1.

Genital primordium. A small mass of cells, not clearly made out, just anterior to the bladder, represents the incipient reproductive system.

Excretory system. The excretory bladder is small and heart-shaped. The island of Cort is small. The tail branch passes down the center of the tail stem to fork at the base of the furcae and the resulting branches empty on the posterior furcal surfaces a short ways in from the tips of the furcae. Two body branches leave the antero-lateral edges of the bladder, pass laterally and anteriorly almost to the acetabulum and there unite in a post-acetabular commissure. A convoluted common collecting tubule enters the inner anterior corner on each side just before the beginning of the commissure. These tubes receive the anterior and posterior collecting tubes near their point of entrance into the bladder branches. The anterior collecting tube connects with two flame cells, one at the anterior level of the pharynx, the other at the anterior level of the acetabulum. The posterior collecting tube divides into two tubules, one connecting with the tail flame cell, the other in turn dividing to connect with two flame cells at the postero-acetabular level, and two at the posterior body limit (Fig. 1). The flame cells are large and conspicuous, measuring 6.3 μ in length.

Tail structures. The tail stem contains seven to eight pairs of large caudal bodies all of about equal size. They are quite distinct in freshly emerged cercariae. The only structure observed is a large clear nucleus. Another pair is located at the base of the furcae, with two smaller ones between them. Several smaller cells occur in the furcae and appear similar to the larger caudal bodies in structure. The tail stem in living individuals tends to be corrugated due to slight contraction. About 40 small nuclei are found on the edges of the stem, one between each ridge. The caudal bodies appear to be attached in some way to the median caudal excretory tube, for during muscular contraction and expansion they move up and down within the tail stem.

Daughter sporocysts. The daughter sporocysts are long slender sacs embedded usually in the digestive gland of the snail as well as in other tissues. Unfortunately the snail died before the live sporocysts could be studied so that details could not be satisfactorily ascertained. The general appearance, however, is quite similar to that of *C. dohema* Cort and Brackett, 1937.

Infection experiments. Several unsuccessful attempts were made to determine the life cycle of this cercaria. Cercariae were placed with mayfly and dragonfly nymphs, gammarids, tadpoles, fishes, and mice.

It seemed as though penetration occurred in the fishes, but no positive results were obtained.

DISCUSSION

Cercaria pseudoburti n. sp. resembles in many respects *C. burti* Miller, 1923 (hence the specific name), *C. dohema* Cort and Brackett, 1937 and *C. ranae* Cort and Brackett, 1938. A careful survey of the literature of strigeid cercariae indicates that these four species are unique in the presence of a post-acetabular excretory commissure. *C. pseudoburti,* in many ways, lies between *C. burti* and *C. dohema.* It resembles *C. burti* in having the same general shape, the same flame cell pattern, about equal size of anterior organ and acetabulum, tail annulation, spination of furcae, length of esophagus and ceca, and number of penetration glands. It differs from *C. burti* in the presence of eyespots, lack of spination on tail stem, expansions of ducts of penetration glands in anterior organ, and absence of distal disjoined parts of intestinal ceca. *C. pseudoburti* resembles *C. dohema,* besides the presence of the post-acetabular commissure, in the presence of eyespots, relatively similar body measurements, and caudal bodies at base of furcae. It differs from *C. dohema* in the nearly equal sucker sizes, long intestinal ceca, eight instead of six penetration glands, expansion of penetration gland ducts in the anterior organ, flame cell pattern, exit of furcal excretory tubes posteriorly rather than anteriorly, and arrangement of spines. It resembles *C. ranae* in the presence of eyespots and post-acetabular excretory commissure; it differs from *C. ranae* in size, presence of caudal bodies, number and position of penetration glands, and shape and size of ceca.

SUMMARY

A new species of strigeid cercaria, *C. pseudoburti,* from *Lymnaea (P.) columella* Say in western Massachusetts, is described in detail and figured. A careful survey of the literature shows that *C. pseudoburti, C. dohema, C. burti,* and *C. ranae* are closely related with respect to the post-acetabular excretory commissure but are specifically different.

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